MicroRNAs:
Novel Biomarkers for Human Cancer

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BACKGROUND: MicroRNAs (miRNAs), small RNA molecules of approximately 22 nucleotides, have been shown to be up- or downregulated in specific cell types and disease states. These molecules have become recognized as one of the major regulatory gatekeepers of coding genes in the human genome.

CONTENT: We review the structure, nomenclature, mechanism of action, technologies used for miRNA detection, and associations of miRNAs with human cancer. miRNAs are produced in a tissue-specific manner, and changes in miRNA within a tissue type can be correlated with disease status. miRNAs appear to regulate mRNA translation and degradation via mechanisms that are dependent on the degree of complementarity between the miRNA and mRNA molecules. miRNAs can be detected via several methods, such as microarrays, bead-based arrays, and quantitative real-time PCR. The tissue concentrations of specific miRNAs have been associated with tumor invasiveness, metastatic potential, and other clinical characteristics for several types of cancers, including chronic lymphocytic leukemia, and breast, colorectal, hepatic, lung, pancreatic, and prostate cancers.

SUMMARY: By targeting and controlling the expression of mRNA, miRNAs can control highly complex signal-transduction pathways and other biological pathways. The biologic roles of miRNAs in cancer suggest a correlation with prognosis and therapeutic outcome. Further investigation of these roles may lead to new approaches for the categorization, diagnosis, and treatment of human cancers.

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Although cancer was once thought to be an acute process leading to imminent death, current knowledge of tumor cell biology suggests that cancer is a chronic condition and that the ongoing development of targeted cancer therapies will continue to increase survival prospects. Human cancers comprise both genetic (inherited and acquired) and epigenetic alterations. Many tumor suppressor genes and oncogenes have been described, and the discovery of new tumor markers continues at a rapid pace *(1)*.

Recently, a novel group of biomarkers, microRNAs (miRNAs),[2] has been discovered. These molecules appear to be cell type and disease specific, unlike most other biomarkers that are currently available. miRNAs promise to have an impact on laboratory medicine as new diagnostic and prognostic markers, as indicators of therapeutic response, and as targets of novel therapies. This review highlights some of what is known about miRNAs and human cancer.

miRNA

MicroRNAs are a family of endogenous, small (approximately 22 nucleotides in length), noncoding, functional RNAs. Bioinformatics approaches for identifying miRNAs rely on evolutionarily conserved sequences *(2)*. It is estimated that there may be 1000 miRNA genes in the human genome (http://www.sanger.ac.uk/Software/Rfam/mirna/). miRNAs are expressed in a tissue-specific manner, and changes in miRNA expression within a tissue type can be correlated with disease status *(3, 4)*.

miRNAs are transcribed by RNA polymerase II or III as longer primary-miRNA molecules, which are subsequently processed in the nucleus by the RNase III endonuclease Drosha and DGCR8 (the "microprocessor complex") to form intermediate stem–loop structures approximately 70 nucleotides long called "precursor miRNAs" (pre-miRNAs) *(5–8)* (Fig. 1). These pre-miRNAs fold to form imperfect stem–loop struc-
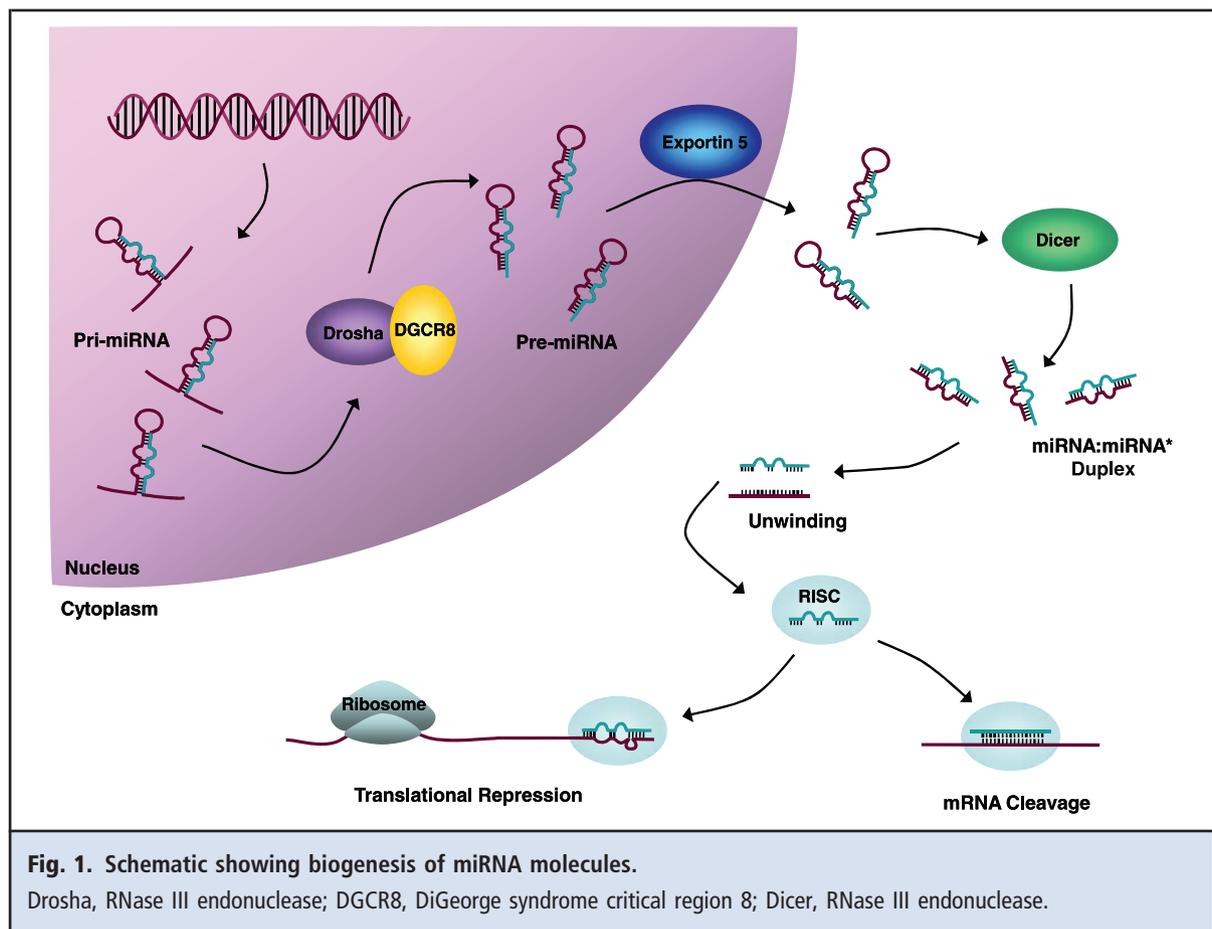
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[2] Nonstandard abbreviations: miRNA, microRNA; pre-miRNA, precursor miRNA; miRNA*, an RNA fragment similar in size to the mature miRNA sequence, with which the former forms an imperfect duplex after Dicer has removed the pre-miRNA loop; T_m, melting temperature; LNA, locked nucleic acid; PDCD4, programmed cell death 4; TPM1, tropomyosin 1; LOH, loss of heterozygosity; AIB1, amplified in breast cancer 1; CLL, chronic lymphocytic leukemia; HCC, hepatocellular carcinoma; NSCLC, non–small cell lung cancer; EGFR, epidermal growth factor receptor; PDAC, pancreatic ductal adenocarcinoma; PanIN, pancreatic intraepithelial neoplasia; CaP, prostate cancer.

Fig. 1. Schematic showing biogenesis of miRNA molecules.
Drosha, RNase III endonuclease; DGCR8, DiGeorge syndrome critical region 8; Dicer, RNase III endonuclease.

tures that are transported with the help of exportin-5 from the nucleus to the cytoplasm, where they undergo further processing by another RNase III endonuclease, Dicer *(9, 10)*. Dicer removes the loop of the pre-miRNA to produce an imperfect duplex made up of the mature miRNA sequence and a fragment of similar size (miRNA*), which is derived from the opposing arm of the pre-miRNA. The miRNA strand of the duplex is loaded onto the RNA-induced silencing complex (RISC); the miRNA* separates from the duplex and is degraded *(11)*.

miRNAs regulate gene expression by regulating mRNA translation and degradation. The mechanism by which they regulate mRNA is dependent on the degree of miRNA complementarity with the mRNA molecule. Perfect (or near perfect) complementarity is thought to target the mRNA for degradation by the RISC, whereas imperfect complementarity is thought to block translation of the mRNA by the ribosome *(12)*.

Identification of miRNA targets has been difficult because only the seed sequence (about 6–8 bases) of the approximately 22 nucleotides aligns perfectly with the target mRNA's 3′ untranslated region *(2, 13)*. The remainder of the miRNA may bind perfectly to the target mRNA, but more often it does not. Bioinformatics approaches can identify putative targets for particular miRNAs through analysis of the miRNA seed sequences *(2)*; however, these miRNAs need to be assayed in vitro or in vivo to determine if they truly affect the proposed mRNA.

Once a sequence has been determined to be a unique miRNA, the miRBase Registry assigns a name according to existing guidelines *(14, 15)*. In the database, a sequence of 3 or 4 letters designates the species (e.g., "hsa" for *Homo sapiens*); however, this prefix is usually dropped in the literature. The core of the miRNA name is the designation "miR" (denoting a mature sequence) followed by a sequentially assigned unique identifying number. Lettered suffixes are added to miRs that differ by only 1 or 2 bases (e.g., miR-10b), and numbered suffixes are assigned to miRs that have the same sequence but are derived from different primary transcripts. A suffix of −5p or −3p is given when mature miRNAs are derived from the 5′ arm or the 3′ arm, respectively, of the precursor miRNA. For this

rapidly growing field, the miRBase registry maintains the latest nomenclature designations.

miRNA-Detection Technologies

There are several methods for detecting miRNAs and/or determining miRNA profiles of particular cell types, such as microarrays, bead-based arrays, and quantitative real-time PCR. The principle of miRNA microarrays is based on the Watson–Crick base pairing of nucleic acids. Microarrays permit the simultaneous detection of hundreds of miRNAs. A set of oligonucleotide capture probes are spotted on glass slides, and a sample of extracted RNA enriched for small-molecule RNAs is allowed to hybridize with the capture probes. Because miRNAs are short, it can be difficult to normalize the melting temperatures (T_m) of the probes across an array without compromising sensitivity or specificity. This problem has been overcome by the use of locked nucleic acids (LNAs). LNAs contain at least one LNA monomer—a nucleic acid analog in which the 2′ oxygen atom and the 4′ carbon atom of the ribose moiety is "locked" by a connecting methylene bridge (16). Each incorporated LNA monomer increases the T_m of the nucleic acid duplex by 2–10 °C (17). Therefore, by adjusting the number of LNA monomers incorporated in a capture probe, all the probes across an array can be T_m-normalized despite the short length of the miRNA. Many disease associations have been discovered on microarrays.

Bead-based arrays, such as the Luminex FlexmiR™ arrays, also permit simultaneous quantification of hundreds of miRNAs (18). Locked Nucleic Acid (LNA™; Exiqon) probes are coupled to carboxylated polystyrene microspheres that incorporate variable mixtures of 2 fluorescent dyes that allow a flow cytometer to identify each microsphere (up to 100) by its unique color. Each microsphere is coupled with an LNA molecule that is specific for a particular miRNA; these probes permit discrimination between closely related members of an miRNA family. Total RNA is extracted from the sample, biotinylated, and then hybridized with the microspheres. The microspheres are washed, incubated with streptavidin–phycoerythrin, and analyzed on a Luminex analyzer. The analyzer can both identify the fluorescent microsphere and measure the intensity of the streptavidin–phycoerythrin fluorescence, allowing the user to see which miRNAs are present in the sample. The assay can also provide quantitative results if a calibration curve is produced with appropriate calibration materials, such as synthetic oligonucleotides.

Quantitative real-time PCR can also be used to detect miRNAs. Quantification of mature miRNAs usually requires reverse transcription of the miRNA with a stem–loop primer (19) (Fig. 2). The cDNA is then used in the real-time PCR reaction. A mixture of forward and reverse primers and a dual-labeled probe (TaqMan®) are used to amplify and detect the cDNA target. The probe has a reporter dye on the 5′ end and a quencher on the 3′ end. If the target sequence is present during the PCR, the probe binds to the target sequence. During the extension stage of the PCR cycle, the reporter dye is released by the 5′ exonuclease activity of *Taq* polymerase, and because the reporter and quencher have been separated, the fluorescence from the reporter dye is detected. Primary RNAs and/or pre-miRNAs can also be quantified with the same methods; however, such assays require adjusting the designs of the primers and probes (19).

miRNA in Breast Cancer

Breast cancer, the second-leading cause of cancer-related deaths in women, is expected to account for 26% of new cancer diagnoses in 2008 (20). Several miRNAs are associated with breast cancer. For example, miR-155 is up-regulated in breast cancer, suggesting that it may act as an oncogene (21, 22). Upregulation of miR-373 and miR-520c promotes metastasis by inhibiting CD44 expression. Increased expression of the CD44 isoform CD44s is associated with overall survival in breast cancer patients (23, 24). Thus, there is an inverse relationship between CD44 expression and the concentrations of miR-373 and miR-520c. Ma et al. (22) showed increased expression of the gene encoding miR-10b, which is upregulated by the transcription factor Twist1. Overproduction of miR-10b can promote tumor invasion in vivo (22). miR-21 has also been found to be upregulated in breast cancer, and this upregulation causes downregulation of 2 important targets: programmed cell death 4 (PDCD4) and tropomyosin 1 (TPM1) (25–27). PDCD4 is a tumor suppressor that targets translation by inhibiting eukaryotic initiation factor 4 (28). TPM1 is a member of the tropomyosin family of proteins, which are associated with actin and serve to stabilize microfilaments (29).

miR-17–5p, also known as miR-91, has been found to be down-regulated in breast cancer (30). miR-17–5p is encoded by a gene located on chromosome 13q31, which is a region that undergoes loss of heterozygosity (LOH) in breast cancer (31). This miRNA normally represses translation of the AIB1 (amplified in breast cancer 1) mRNA (30). AIB1 is a coactivator of the cell cycle regulator E2F1, and it also enhances estrogen receptor–dependent transcription (32, 33). Along with miR-20a, miR-17–5p also nega-
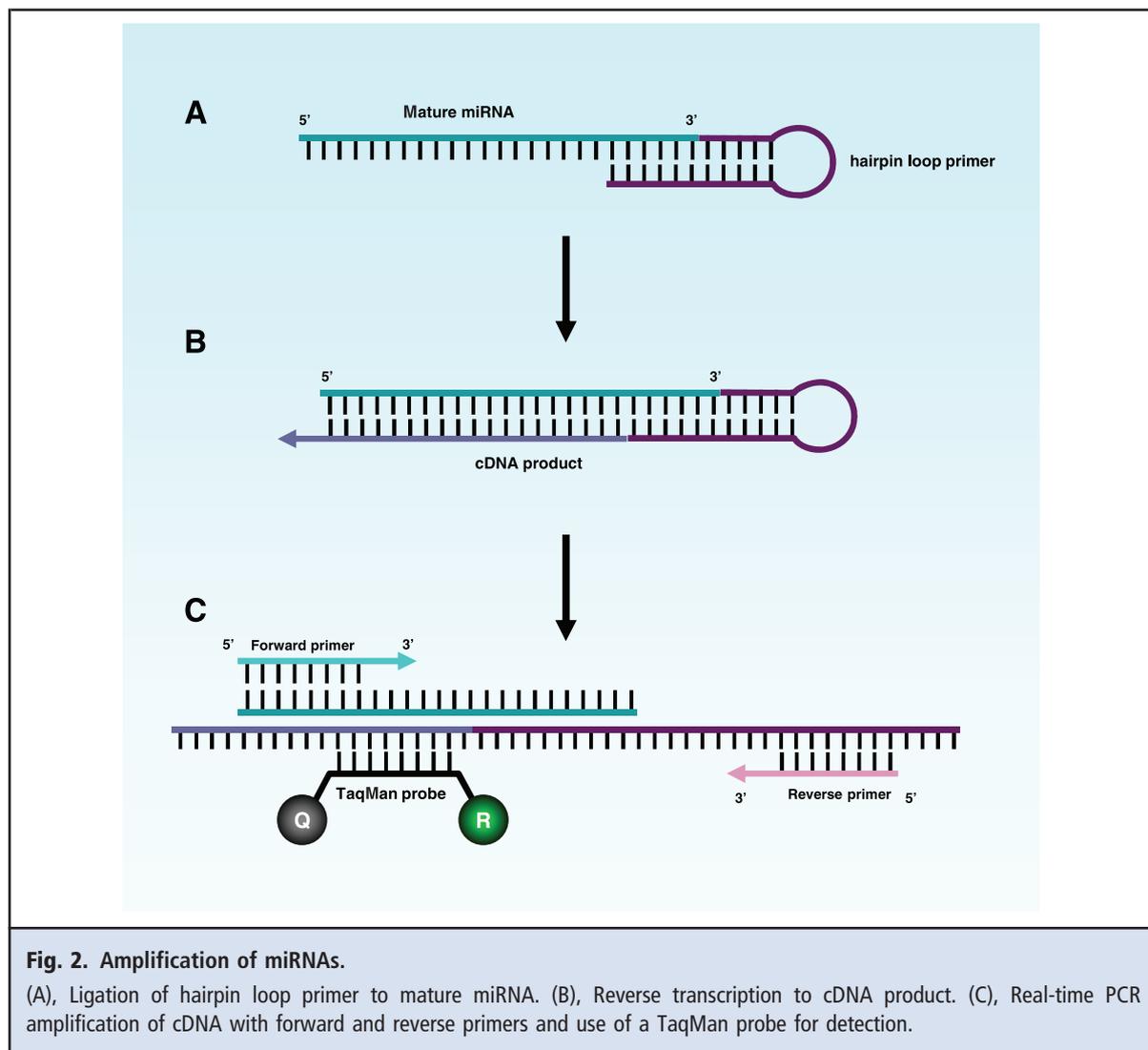
Fig. 2. Amplification of miRNAs.

(A), Ligation of hairpin loop primer to mature miRNA. (B), Reverse transcription to cDNA product. (C), Real-time PCR amplification of cDNA with forward and reverse primers and use of a TaqMan probe for detection.

tively regulates the *CCND1*[3] (cyclin D1) gene *(34)*. Patients with low miR-335 and miR-126 concentrations have been observed to have shorter median times to metastatic relapse *(35)*. Downregulation of miR-335 promotes metastasis by upregulating the extracellular matrix protein tenascin C and the transcription factor SOX4 *(35)*. The role of miR-126 seems to be that of a tumor suppressor, and a decreased concentration of this miRNA promotes cell proliferation *(35)*.

Differential expression of genes encoding some miRNAs seems to be associated with particular pathologic features of breast cancer. For example, expression of the gene encoding miR-30 seems to correlate with estrogen receptor and progesterone receptor status; downregulation of this miRNA is found in estrogen receptor– and progesterone receptor–negative tumors *(21)*. miR-213 and miR-203 appear to correlate with tumor stage; increased expression of the genes encoding these miRNAs is found in higher-stage tumors *(21)*. miR-206 has been found to target the 3′ untranslated region of the estrogen receptor α protein, leading to an inverse correlation between miR-206 concentration and estrogen receptor α status *(36, 37)*.

miRNA in Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, and the disease has a very variable clinical outcome. There is great in-

terest in developing better prognostic tests for this disease. Some studies have tried to correlate the concentrations of miRNAs in CLL cells with other biomarkers, such as ZAP-70 status, IgV_H mutation status, or 13q14 deletion status; however, a consensus has yet to be reached on this issue (38, 39). miR-150 is upregulated in CLL blood samples along with miR-155, whose gene maps to the last exon of the B-cell integration cluster and is upregulated in CLL cells compared with nonpathologic B cells (38, 40, 41).

Many miRNAs have been shown to be downregulated in CLL. Reduced expression of the genes encoding miR-15a and miR-16-1 correlates with a good prognosis; these genes are found at chromosome 13q14.3, which is deleted in 68% of CLL patients. Some uncertainty exists, however, as to the target(s) of these miRNAs (39, 40, 42, 43). Although results of transfecting a megakaryocytic cell line with these 2 miRNA genes have shown that the miRNAs may target the antiapoptotic protein BCL-2, the observation has also been made that changes in the expression of these 2 genes do not significantly correlate with changes in BCL2 (B-cell CLL/lymphoma 2) gene expression in CLL patients (40, 42). Several other targets for miR-15a and miR-16-1 are currently under investigation (43). The genes encoding miR-92 miRNAs, which are members of the miR-17-92 gene cluster that maps to 13q31–32, are downregulated in CLL (40). Genes encoding miR-181 and miR-29 miRNAs that target the TCL1A (T-cell leukemia/lymphoma 1A) gene are downregulated in CLL (39, 44). It is possible that reduced expression of the genes encoding miR-181a-2 [and MIRLET7A1 (microRNA let-7a-1), which is also downregulated in CLL] may be due to impaired processing of the precursor molecule (45). Low concentrations of miR-29 miRNAs correlate with poor prognosis in CLL (39). Reduced expression of the genes encoding miR-143 and miR-145 has also been observed (46).

miRNA in Colorectal Cancer

Colorectal cancer is the third-leading cause of cancer-related deaths, and an estimated 150 000 new cases will be reported this year (20). As with other human cancers, several miRNAs are up- or downregulated in this tumor type (47). miR-31, miR-96, miR135b, and miR-183 have been found to be upregulated in colorectal neoplasms; the transcription factor CHES1 (which is involved in repressing apoptosis) is a potential target of miR-96. High expression of the gene encoding miR-21 correlates with reduced expression of the gene encoding tumor suppressor protein PDCD4; miR-21 may also target the same genes as in breast neoplasms [PTEN, phosphatase and tensin homolog; TPM1, tropomyosin 1 (alpha)]. miR-135a and miR-135b are up-

regulated, and this upregulation correlates with reduced expression of the APC (adenomatous polyposis coli) gene (48).

miR-143 and miR-145 are both downregulated in colorectal cancer, similar to CLL. The genes encoding these miRNAs are both located on 5q23, and these miRNAs possibly originate from the same primary miRNA (49, 50). miR-126 promotes cell proliferation through modulation of phosphatidylinositol 3-kinase signaling (51). miR-133b is also downregulated, and one of its putative targets is KRAS (52). KRAS is a member of the Ras family of proteins, which regulates signaling pathways involved in cellular proliferation, differentiation, and survival. In addition, Lanza et al. are investigating whether a combined miRNA/mRNA panel is able to distinguish between microsatellite-stable and -unstable colorectal cancers (53).

miRNA in Hepatocellular Cancer

Hepatocellular carcinoma (HCC) is the fifth-leading cause of cancer-related death in men in the US, with an estimated 21 000 new cases (men and women) diagnosed on an annual basis (20). The major etiologies of HCC include viral infection, metabolic abnormalities, and immune-related disorders. Five-year survival rates approach 50%–70%, with local recurrence rates of >70% at 5 years (54, 55). Neoplasms of the liver are clinically heterogeneous and have associated risk factors and genetic alterations. The genes for specific miRNAs have been shown to be aberrantly expressed in various liver tissues that include HCC.

Murakami et al. were the first to use microarray technologies to profile miRNA gene expression in HCC (56). They analyzed miRNA gene expression profiles in 25 pairs of HCC and adjacent nontumor tissues as well as in 9 chronic hepatitis samples. In this study, the genes for 3 miRNAs (miR-224, miR-18, and pre–miR-P18) exhibited higher expression in the HCC samples than in nontumor tissues, and the genes for 5 other miRNAs (miR-199a, miR-199a*, miR-200a, miR-125a, miR-195) showed lower expression in the HCC samples than in the adjacent nontumor tissue samples. With these 8 miRNAs, Murakami et al. developed an overall prediction accuracy of 97.8% (56).

In a more recent study, Ladeiro et al. demonstrated the utility of miRNA profiling for distinguishing benign from malignant hepatocellular tumors (57). This study also characterized miRNAs in several subgroups of tumors on the basis of the presence of oncogene and tumor suppressor gene mutations and specific risk factors. Both benign and malignant hepatocellular tumors showed increased expression of the gene encoding miR-224 and decreased expression of those encoding miR-122a and miR-422b. HCCs had

increased concentrations of miR-21, miR-10b, and miR-222, whereas benign tumors showed decreased expression of the genes encoding miR-200c and miR-203. Of the HCC cases, those associated with alcohol consumption showed decreased miR-126 concentrations, whereas the cases associated with hepatitis C virus infection showed upregulation of miR-96. These findings have important implications for our understanding of liver pathophysiology and could shed light on novel therapeutic approaches.

Li et al. identified a profile of 69 miRNAs that differentiated noncancerous from cancerous liver tissues (58). Eight of these miRNAs were chosen for further validation to distinguish between benign and malignant liver tumors, as well as to differentiate healthy liver tissue. miR-125b was shown to be downregulated in HCC. Overexpression of the gene for miR-125b was associated with good survival in HCC patients. In HCC patients, Jiang et al. identified genes for 19 miRNAs whose expression was associated with either poor survival (low expression) or good survival (high expression) (59).

Li et al. postulated that the mechanism of action of miR-125b involved inhibition of cell proliferation by suppressing phosphorylation and thus inactivation of Akt (58). Akt is the most crucial downstream signaling mediator of phosphatidylinositol 3-kinase. In addition, Fornari et al. established a potential oncogenic function of miR-221, which is upregulated in HCC (60). miR-221 targets the cyclin-dependent kinase inhibitors CDKN1B/p27 and CDKN1C/p57. Upregulation of miR-221 causes a downregulation of these inhibitors and promotes loss of cell cycle control.

miRNA in Lung Cancer

In 2008, 215 000 new cases of lung cancer will have been diagnosed and 162 000 individuals will have succumbed to their lung disease (20). Non–small cell lung cancer (NSCLC) is the most common cause worldwide of lung cancer–related deaths. Many studies at the molecular level have identified gene mutation spectra and gene expression profiles associated with biological processes that are altered in lung carcinogenesis (61, 62). Given that miRNAs have been shown to play key roles in carcinogenesis by regulating the translation and degradation of specific mRNAs controlling cellular processes, there is also a potential for miRNAs to be valuable biomarkers of lung cancer.

The lethal-7 (let-7) gene was first identified as playing a critical role in the development of *Caenorhabditis elegans* and was later shown to have homologs in the human genome (63). In *C. elegans*, the let-7 miRNA family negatively regulates let-60/RAS. Johnson et al. have shown that the 3′ untranslated regions of the human RAS genes contain many let-7–complementary sites, and in lung cancer, the expression of let-7–related genes is 50% lower than in healthy tissue (64). The concentration of RAS protein, on the other hand, is significantly higher, suggesting a mechanism for let-7 homologs in human lung cancer. Kumar et al. showed that *MIRLET7G* (microRNA let-7g)-expressing NSCLCs also had reduced concentrations of the RAS and HMGA2 proteins (65). Loss of miRNA control of RAS expression could thus lead to RAS overproduction and contribute to the formation of a human cancer.

LOH of chromosome 3p is one of the most frequent genetic events in lung carcinogenesis. Weiss et al. showed that loss of the gene encoding miR-128b (located on chromosome 3p) correlated with the response to targeted inhibition of epidermal growth factor receptor (EGFR) (66). LOH of miR-128b can be considered equivalent to losing a tumor suppressor gene, because it permits increased production of EGFR. Weiss et al. initially showed that miR-128b is a regulator of EGFR in NSCLC cell lines and determined that miR-128b directly regulates EGFR (66). miR-128b LOH was frequent in tumor samples, and this LOH was significantly correlated with the clinical response to and survival after gefitinib therapy. Yu et al. identified a signature of 5 miRNAs (let-7a, miR-221, miR-137, miR-372, miR-182) that predicted treatment outcome in NSCLC patients (67). Patients with a high risk score for these 5 miRNAs had an increased relapse rate and shortened survival times.

miRNA in Pancreatic Cancer

Pancreatic cancer is the fourth-leading cause of cancer-related deaths in the US, with a 5-year survival rate of <5%. Approximately 38 000 new cases and 34 000 pancreatic cancer–related deaths will have occurred in the US in 2008 (20). Eighty-five percent of pancreatic tumors originate from the epithelial lining of the pancreatic duct [pancreatic ductal adenocarcinoma (PDAC)] (68). The mortality and morbidity associated with this disease correspond to the overall poor prognosis of pancreatic cancer, which is due to the late clinical presentation, its aggressive invasive and metastatic potential, and its resistance to chemotherapy and radiation therapy. Despite advances in the clinical management of pancreatic cancer, there is currently a lack of effective biomarker-based strategies for early detection of pancreatic cancer or for differentiating between PDAC and benign disease, such as chronic pancreatitis. To identify more accurate and sensitive biomarkers for pancreatic cancer, researchers have begun to describe miRNA profiles that might be able to provide an earlier diagnosis.

In several studies, miR-216 was identified as being specific for the pancreas (69–71). Other studies have identified abnormal production of as few as 2 miRNAs, miR-196a and miR-217, that can distinguish PDAC samples from samples of healthy pancreas and chronic pancreatitis (72). In a later study, increased production of miR-196a was determined to predict poor survival of patients with PDAC (73). Although early-stage pancreatic carcinoma can be treated surgically, most cases present at an advanced stage, when surgical resection is not possible because of the vascular dissemination of the tumor and its spread to regional lymph nodes. Szafranska et al., therefore, evaluated the utility of miRNA gene expression profiles in fine-needle aspirate samples for reliably identifying the status of pancreatic tissue disease and to distinguish benign from malignant pancreatic tissues (74). Differences between miR-196a and miR-217 in raw threshold cycle values obtained in real-time PCR assays clearly separated malignant and benign tissues, for both frozen samples and fine-needle aspirates. The gene encoding miR-217 was highly expressed only in healthy pancreatic tissues, and miR-196a was detected above background only in PDAC samples. Because healthy pancreas consists of about 90% acinar cells, these observations suggest that miR-217 is primarily produced in acinar cells. miR-196a was found to be produced only in ductal adenocarcinoma cells, and not in healthy acinar or ductal cells.

In addition, Szafranska et al. have shown that there is an absence of miR-196a production in pancreatic intraepithelial neoplasia 1b (PanIN-1b) lesions from microdissected pancreatic tissues; such lesions are likely to have a low propensity to progress into PDAC (74). Sixty percent of more aggressive and advanced PanIN-3 lesions were positive for miR-196a, whereas the relative level of miR-196a gene expression dropped to 25% in the intermediate PanIN-2 lesions.

miRNA in Prostate Cancer

Prostate cancer (CaP) is the most frequently diagnosed malignant tumor and the second-leading cause of cancer deaths in American men. It is estimated that in 2008 there will have been more than 186 000 newly diagnosed CaP cases and more than 28 000 attributed deaths (20). The mechanisms that underlie the occurrence and progression of CaP remain largely unknown. Some aberrantly produced miRNAs have been discovered in CaP cell lines, xenografts, and clinical samples

(75). These miRNAs may play critical roles in the pathogenesis of CaP.

Porkka et al. identified 51 individual miRNAs from an expression profile of 319 genes encoding miRNAs as being either up- or downregulated in CaP compared with healthy tissue (76). Twenty-two of these miRNAs were downregulated in all CaPs examined, and 15 were downregulated only in the hormone-refractory cancers. Similarly, these investigators showed 8 miRNAs to be upregulated in all CaPs, but only 6 were upregulated in the hormone-refractory cancers. Ozen et al. have also evaluated miRNA expression in CaP and demonstrated miRNA gene expression profiles consistent with the disease process (77). In this study, downregulated miRNAs were known to have target mRNAs, including those for RAS, E2F3, BCL-2, and MCL-1.

Conclusion

Despite many years of effort to identify biomarkers for human cancers, no biomarker has generated the excitement that has accompanied the interest in the potential of miRNAs. The biologic roles of miRNAs suggest correlations with prognosis and therapeutic outcome. Inhibition of miRNAs with "antagomirs" is an attractive direction for therapy. There are numerous questions outstanding that could lead to further applications of these biomarkers. For example, what are the expression levels of miRNA genes in primary, second primary, or metastatic tumors in any given patient? If similar miRNAs are up- or downregulated in different tumor types, what are the downstream targets of these miRNAs, and are these targets similar or different for different tumor types? Answers to these questions may help shape the way human cancers are categorized, diagnosed, and treated in the future.

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