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Review

# Principles of microRNA involvement in human cancers

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**Abstract**

Naturally occurring microRNAs (miRNAs), small non-coding RNAs of 19 to 24 nucleotides (nt), are encoded in the genomes of invertebrates, vertebrates, and plants. miRNAs act as regulators of gene expression during development and differentiation at the transcriptional, posttranscriptional, and/or translational levels, although most target genes are still elusive. Many miRNAs are conserved in sequence between distantly related organisms, suggesting that these molecules participate in essential processes. In this review, we present principles related to the basic and translational research that has emerged in the last decade, a period that can be truly considered the "miRNA revolution" in molecular oncology. These principles include the regulation mechanism of miRNA expression, functions of miRNAs in cancers, diagnostic values and therapeutic potentials of miRNAs. Furthermore, we present a compendium of information about the main miRNAs that have been identified in the last several years as playing important roles in cancers. Also, we orient the reader to several additional reviews that may provide a deeper understanding of this new and exciting field of research.

**Key words** MicroRNA, non-coding RNA, cancer, mutation, biomarker

Structurally, microRNAs (miRNAs) are 19- to 24-nucleotide (nt) RNAs processed from much longer primary transcripts (hundreds to thousands of nucleotides) that arise from hairpin loop structures (60- to 110-nt) after successive enzymatic maturation steps by the ribonucleases Drosha in the nucleus and Dicer in the cytoplasm[1].

Functionally, miRNAs regulate gene expression in a sequence-specific fashion. Initially transcribed by RNA polymerase II as long, capped, polyadenylated primary miRNAs (pri-miRNAs), miRNAs undergo a complex processing mechanism. First, the double-stranded RNA-specific ribonuclease Drosha, in conjunction with its binding partner DiGeorge syndrome critical region gene 8 (DGCR8, or Pasha), processes pri-miRNAs into hairpin RNAs of 60- to 110-nt known as pre-miRNAs. Translocated from the cell nucleus to the cytoplasm by Exportin 5, pre-miRNA is processed by a ribonuclease III (Dicer) and transactivating response RNA-binding protein (TRBP, which binds human immunodeficiency virus 1) into an 18- to 24-nt duplex. Finally, the duplex interacts with a large protein, RNA-induced silencing complex (RISC), which includes argonaute proteins (AGO1-4 in humans). One strand of the miRNA duplex remains stably associated with RISC and becomes a mature miRNA, which guides the RISC complex mainly (but not exclusively) to the 3'-untranslated region (3'-UTR) of target mRNAs. Consequently, the translation and/or stability of mRNAs are impaired, causing a reduction in protein expression levels[2].

Evidences are emerging that miRNAs' effects on gene expression may be more varied than initially proposed. For example, miRNAs can activate rather than suppress mRNA expression in particular cell cycle conditions. Upon cell cycle exit, *miR-363-3* and *let-7*, via recruitment and modification of specific micro-ribonucleoproteins (RNPs) such as AGO2 and

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fragile X mental retardation autosomal homolog 1 (FXR1) to AU-rich elements inside mRNA 3'-UTRs, can turn on the translation of proteins that they normally repress during cell proliferation [3]. Furthermore, miRNA-induced mRNA repression was also found to occur via binding sites located inside mRNA-coding sequences, as shown for miRNAs regulating fundamental processes such as embryonic stem cell differentiation [4]. Additionally, specific miRNAs that carry a distinct hexanucleotide terminal motif, such as *miR-29b*, were found to be enriched in the cell nucleus, suggesting extra miRNA functions in different subcellular compartments [5]. It has been shown that miRNAs in the nucleus may act at the promoter level, affecting transcription; for example, *miR-373* binds to the cadherin 1 (*CDH1*) promoter and stimulates transcription [6].

Finally, miRNAs can directly interact with proteins; for example, the interaction of *miR-368* with heterogeneous nuclear ribonucleoprotein (hnRNP) E2 is independent of the miRNA seed sequence, and this interaction leads to the release of *CCAAT*/enhancer binding protein alpha (*CEBPA*) mRNA from hnRNP E2-mediated translational inhibition [7]. Because each miRNA has hundreds or thousands of targets and the full coding genome is probably under the control of miRNAs, miRNAs may be involved in any type of physiologic process and pathway, such as B-cell lineage fate (*miR-181*), B-cell survival (*miR-15a* and *miR-16-1*), cell proliferation control (*miR-125b* and *let-7*), brain patterning (*miR-430*), pancreatic cell insulin secretion (*miR-375*), and adipocyte development (*miR-145*) [8].

In this review, we summarize the main principles of miRNA involvement in human cancers. These principles are important not only for scientists in general but also for oncologists, as the field of non-coding RNAs (ncRNAs) has already been shown to touch every aspect of human oncology. We also briefly introduce other categories of ncRNAs that are important in human cancers.

## Principles of miRNA Involvement in Human Cancers

### Regulation mechanisms of miRNA expression

***miRNA alterations are ubiquitous*** miRNAs are involved in the pathophysiology of all types of human tumors, both benign and malignant. miRNAs differentially expressed between tumors and normal tissues have been identified in lymphomas, breast cancers, lung cancers, papillary thyroid carcinomas, glioblastomas, hepatocellular carcinomas, pancreatic tumors, pituitary adenomas, cervical cancers, brain tumors, prostate cancers, kidney and bladder cancers, and colorectal cancers [9]. Furthermore, miRNA alterations have been identified in other human diseases, including cardiac and autoimmune disorders and psychiatric conditions such as schizophrenia [10,11]. The development of high-throughput profiling methods to detect miRNA expression in human tissues has provided invaluable tools to investigate the roles of miRNAs in both physiologic and pathologic conditions (Table 1) [12]. Data exponentially accumulated in the last 9 years clearly show that miRNA alterations play a critical role in cancer initiation and progression (Table 2) [13]. Recently, investigators using high-throughput profiling techniques observed a link in cytogenetically well-defined chronic lymphocytic leukemia (CLL) samples between the level of expression of *miR-34a*, a member of a miRNA family positively regulated by tumor protein 53 (*TP53*) gene, and the level of response to DNA damage, the TP53 status, and significantly, the degree of response to fludarabine-based treatment. Low *miR-34a* expression level was significantly associated with impaired DNA damage response, *p53* mutations, and resistance to fludarabine, either with or without *p53* deletion. Up-regulation of *miR-34a* expression after irradiation was associated with induction of B-cell lymphoma-associated X protein (Bax) and P21 expression but not P53–up-regulated modulator of apoptosis (Puma) expression [14]. These findings are straightforward and provide a new piece to the recently identified puzzle of miRNA involvement in drug resistance and sensitivity in patients with CLL.

***Multiple mechanisms causing abnormal miRNA expression*** The main mechanism of the microRNoma (a term we coined for the full complement of miRNAs present in a genome) alteration in cancer cells is represented by aberrant gene expression, characterized by abnormal expression of mature and/or precursor miRNA sequences in comparison with corresponding normal tissues. This abnormal expression is caused by various mechanisms that can act independently or in combination, such as localization of miRNAs at cancer-associated genomic regions (CAGR) [15], epigenetic regulation of miRNA expression [16], or development of abnormalities in miRNA-processing genes and proteins, including mutations in Dicer, TRBP, and Exportin 5 [17]. In various types of tumors, sometimes multiple mechanisms account for the deregulation of a specific miRNA. For example, the tumor suppressor *miR-34a* is positively controlled by TP53 [18], is kept in check by Myc [19], is silenced by aberrant CpG methylation [20], and is located at 1p36 [21], a chromosomal region frequently lost in neuroblastomas. Accordingly, numerous genetic studies have identified miRNA abnormalities in human cancers by dissecting their transcriptional regulators [19,21,22]. Cancer-associated miRNAs have been located downstream of major oncogenes and tumor suppressor genes (TSGs)

**Table 1. Main microRNA-profiling technologies used at the time of this writing[12]**

| Type | Principle | Advantages | Disadvantages |
|---|---|---|---|
| miRNA microarray | miRNA chips are fabricated on polymer-coated surface of glass slides with mature miRNA gene-specific oligonucleotide probes. The chemical covalent-immobilized probes hybridize with biotin-labeled cDNA targets. The signal of the probe-target complex is amplified by staining of Streptavidin Alexa 647 conjugates that affinity bind to biotins of the probe-target complex and are detected by laser scanning. | High-throughput miRNA expression genome-wide profiling is concomitantly achieved on large sample collections processed in parallel using standardized procedures and conditions for data comparison. | Probes on solid substrates affect target hybridization kinetics and discrimination in detecting differences between the 5′ and 3′ ends of highly similar miRNAs in tissues. The dynamic range of microarray data is relatively compressed, about 2.5 orders of magnitude compared with other techniques. |
| Bead-based technology | Polystyrene beads coated with antisense oligonucleotide probes hybridize with biotin-labeled PCR amplicon dsDNA as targets. Staining with Streptavidin phycoerythrin is followed by bead flow cytometry for signal detection. | Solution-phase probe/target hybridization kinetics, which allows for high discrimination among closely related miRNA sequences | Low-throughput profiling for subsets of miRNA to be analyzed per experiment. More bias could be introduced in sample preparation by enrichment, adaptor ligation, and PCR steps. Competitive hybridization between probes and targets of double-stranded PCR amplicons occurs. |
| Stem-loop qRT-PCR for mature miRNA | Stem-loop primer cDNA reverse transcription followed by quantitative TaqMan-based real-time PCR | High sensitivity and specificity; reliable quantitative results, useful in confirming microarray results; low cost | Low-throughput profiling for only a subset of miRNAs |
| qRT-PCR for precursor miRNA | Precursor gene-specific primers cDNA synthesis followed by quantitative real-time PCR | High sensitivity; high specificity; quantitative data | Low-throughput profiling for only a specific subset of miRNAs |
| Next generation sequencer | Genome-wide sequencing of size-selected small RNA | Ultra-throughput for miRNA and small ncRNA profiling and discovery | Data analysis is challenging. |

miRNA, microRNA; cDNA, complementary DNA; PCR, polymerase chain reaction; dsDNA, double-stranded DNA; qRT-PCR, quantitative real-time PCR.

with transcription factor activities; for example, TP53 inhibits tumorigenesis via the transcription of all members of the *miR-34* family [23], and Myc promotes tumorigenesis by both positively and negatively regulating the transcription of different miRNAs (e.g., *miR-17~92* cluster and *let-7* family, respectively) [19]. Similarly, transcription factors that govern the programming of metastatic gene expression have been found to regulate miRNAs. For example, the pleiotropic transcription factor TWIST1 transactivates the prometastatic *miR-10b* [24], and SMAD4, which is downstream of transforming growth factor beta (TGF-β) signaling, activates *miR-155* [25].

## Functions of miRNAs in human cancers

***miRNAs as both oncogenes and tumor suppressor genes*** Various mechanisms allow miRNAs to act as either oncogenes (such as *miR-21* and *miR-155*, which cause acute B-cell leukemia in transgenic mice models) or as TSGs (such as the *miR-15a/16-1* cluster, whose deletion causes CLL in knockout mice). In particular, while some miRNAs act mainly as TSGs, other miRNAs are frequently overexpressed in human cancers and target TSGs, thereby exerting a tumorigenic function. The *miR-15a/16-1* cluster, for instance, controls the expression of about 14% of all genes in the human genome [26], and these miRNAs act as TSGs in CLL by targeting the antiapoptotic gene *Bcl-2* [27]. miRNAs with well-established roles as oncogenes include the *miR-17~92* cluster, which is transactivated by the *c-Myc* oncogene and dramatically accelerates lymphomagenesis in murine models [28,29]; *miR-155*, which induces leukemia in transgenic murine models [30] and has an important function as a regulator of inflammation and immune response [31-33]; and *miR-21*, which targets important TSGs

**Table 2. MicroRNA deregulation in human cancers[13]**

| Human microRNA | Putative function/involved pathways | Deregulation in tumors | Molecular mechanisms and targets | Diagnostic and prognostic markers |
|---|---|---|---|---|
| *let-7* family | Antitumorigenic:<br>-Self-sufficiency in growth signals<br>-Insensitivity to antigrowth signals<br>-Angiogenesis | -Down-regulation in lung, breast, gastric, ovarian, prostate, and colon cancers, CLL, and leiomyomas<br>-Down-regulation of *miR-98* in head and neck cancer cells<br>-Point mutation in the *let-7e* precursor sequence affects maturation | Molecular mechanism:<br>-Represses cell proliferation/ growth<br>*-let-7f* promotes angiogenesis<br>Targets: CCND1, CDC25a, CDK6, CRD-BP, HOXA9, IMP-1, MYC, RAS, TLR4 | Poor prognosis:<br>*-let-7a-2* low expression in lung and ovarian cancers<br>*-let-7b* discriminates high-risk uveal melanomas<br>Drug resistance:<br>*-let-7i* affects chemotherapy potency<br>Therapy:<br>-intranasal delivery of *let-7a* adenovirus reduces growth of Ras-induced lung tumors in mice |
| | Oncogenic:<br>-Self-sufficiency in growth signals<br>-Evasion from apoptosis | -Hypomethylation of *let-7a-3* in lung adenoma carcinomas<br>-Overexpression in AML | Molecular mechanism:<br>*-let-7a* represses NF2 and decreases chemotherapy-induced apoptosis *in vitro* | |
| *miR-16-1/15a* cluster (13q14.3, intron 4 ncRNA DLEU2) | Antitumorigenic:<br>-Self-sufficiency in growth signals<br>-Evasion from apoptosis | -Down-regulation in CLL, DLBCLs, multiple myeloma, pituitary adenoma, and prostate and pancreatic cancers<br>-Germline mutations in B-CLL patients and NZB mouse strain | Molecular mechanism:<br>-Induce apoptosis in leukemia and prostate cancer cells<br>*-miR-16* regulates cell cycle by downregulation of G0/G1 proteins<br>Targets: ACVR2A (X. tropicalis), BCL2, CARD10, CCND1, CDK6, CDC27, CGI-38, DMTF1, MCL1, NGN2, VEGF, WNT3A | Poor prognosis:<br>*-miR-15a* and *miR-16* high expression in *de novo* aggressive CLL<br>Drug resistance:<br>*-miR-16* affects chemotherapy potency, and modulates sensitivity to vincristine in gastric cancer cell lines |
| *miR-17/18a/19a/ 20a/92* cluster (13q31.3, intron 3 C13orf25) | Oncogenic:<br>-Insensitivity to antigrowth signals<br>-Angiogenesis | -Overexpression in lung cancers and lymphomas | Molecular mechanism:<br>*-miR-17, -18a, -19a, -20a*, and *-19b-1* accelerate tumor growth and increase tumor vascularization<br>*-miR-20a* has an antiapoptotic role-lymphoproliferative disease and autoimmunity in transgenic *miR-17/92* cluster mice with increased expression in lymphocytes<br>Targets:<br>AIB1 AML1, BIM1, CTGF, CDKN1A, E2F1, E2F2, E2F3, PTEN, TGFBR2, TSP1, Rb2/P130 | |
| | Antitumorigenic:<br>-Self-sufficiency in growth signals | -LOH of *miR17/92* locus in ovarian (16.5%), breast carcinoma (21.9%), and melanoma (20%) | Molecular mechanism:<br>*-miR-17* reduces proliferation in breast cancer cells<br>Targets: AIB1 | |

**Table 2. MicroRNA deregulation in human cancers[13] (*continued*)**

| Human microRNA | Putative function/involved pathways | Deregulation in tumors | Molecular mechanisms and targets | Diagnostic and prognostic markers |
|---|---|---|---|---|
| *miR-21* (17q23.1, 3´-UTR TMEM49) | Oncogenic:<br>-Self-sufficiency in growth signals<br>-Evasion from apoptosis<br>-Invasion and metastasis | -Overexpression in glioblastomas and breast, lung, prostate, colon, gastric, esophageal, and cervical carcinomas, uterine leiomyosarcoma, and DLBCL | Molecular mechanism:<br>*-miR21* knockdown induces apoptosis in glioblastoma, hepatocarcinoma, lung and breast cancer cells<br>*-miR-21* modulates *K-Ras*-dependent lung tumorigenesis; *miR-21* induces invasion and metastasis in colorectal cancers<br>Targets: BCL2, MASPIN, PDCD4, PTEN, TPM1, RECK, SERPINB5 | Poor prognosis:<br>*-miR-21* high expression in colon and breast cancers<br>Good prognosis:<br>*-miR-21* high expression in *de novo* DLBCL<br>Drug resistance:<br>*-miR-21* affects chemotherapy potency in NCI60 cells |
| *miR-29* family (various) | Antitumorigenic:<br>-Self-sufficiency in growth signals<br>-Invasion and metastasis | -Down-regulation in CLL and colon, breast, and lung cancers and in cholangiocarcinoma tumor models (KMCH) | Molecular mechanism:<br>*-miR-29* family reverts aberrant methylation in lung cancer<br>*-miR-29* activates *p53* and induces apoptosis<br>Targets: DNMT3A and B, DNMT1, MCL1, TCL1, CDK6, p85$\alpha$, CDC42 | Poor prognosis:<br>*-miR-29c* low expression correlates with short interval from diagnosis to therapy in CLL |
| *miR-34* family (1p36.23, and 11q23.1, intergenic) | Antitumorigenic:<br>-Self-sufficiency in growth signals<br>-Insensitivity to antigrowth signals<br>-Evasion from apoptosis<br>-Limitless replicative potential | -Down-regulation in pancreatic cancer cell lines<br>-Hypermethylation of *miR34b/c* in colon cancer | Molecular mechanism:<br>*-miR-34a* induces up-regulation of TP53 pathway and down-regulation of E2F pathway in colon cancer cell lines<br>Targets: BCL2, CCND1, CCNE2, CDK4/6, DLL1, E2F3, Notch1, MYCN, MET, HMGA2, SIRT1 | |
| *miR-143/145* cluster (intergenic, 5q32); | Antitumorigenic:<br>-Self-sufficiency in growth signals | -Down-regulation in colon adenomas and carcinomas, breast and lung cancers, cervical cancer, and B-cell malignancies | Molecular mechanism:<br>*-miR-143* and *miR-145* precursors are abnormally processed in colon cancer<br>Targets: ERK5, HOXA9, PARP8 | |
| *miR-155* (21q21.3, exon 3 ncRNA BIC) | Oncogenic:<br>-Evasion from apoptosis | -Overexpression in pediatric BL, Hodgkin's disease, primary mediastinal lymphomas, and DLBCL and in breast, lung, colon, and pancreatic cancers | Molecular mechanism:<br>-Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in *miR-155* transgenic mice<br>Targets: AGTR1, AID, TP53INP1, RHOA | Poor prognosis:<br>*-miR-155* high expression in lung cancer, DLCBL, and aggressive CLL |
| *miR-181* family (various) | Oncogenic/Antitumorigenic:<br>- Self-sufficiency in growth signals | -Overexpression in breast, pancreatic, and prostate cancers | Molecular mechanism:<br>-MYCN regulates the transcription of *miR-181* cluster<br>Targets: HOXA11, TCL1, ESR1 | Poor prognosis:<br>*-miR-181* low expression in aggressive CLL with 11q deletions<br>*-miR-181a* high expression correlates with short interval from diagnosis to therapy in CLL |
| *miR-221/222* cluster (Xp11.3, intergenic) | Oncogenic:<br>-Insensitivity to antigrowth signals, angiogenesis | -Overexpression in CLL, thyroid papillary carcinoma, and glioblastoma<br>-Down-regulation in AML | Molecular Mechanism:<br>-Promotes cancer cell proliferation<br>*-miR-221/222* impair TRAIL-dependent response<br>-Overexpression contributes to liver tumorigenesis<br>Targets: c-KIT, P27/CDKN1, PTEN | |

CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; NF2, neurofibromatosis type 2; ncRNA, non-coding RNA; DLBCL, diffuse large B-cell lymphoma; B-CLL, B-cell CLL; NZB, New Zealand black; LOH, loss of heterozygosity; 3´-UTR, 3´-untranslated region; E2F, E2 transcription factor; BL, bone lymphocyte; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TGF-$\beta$, transforming growth factor beta.

such as *PTEN*[34] and programmed cell death 4 (*PDCD4*) in several neoplasms[35-37]. In some instances, the same miRNA acts as an oncogene in one type of cells and as a TSG in others because the targets and mechanisms of action differ. For example, *miR-221* is hyperexpressed in liver cancers because the TSG *PTEN* is targeted[38], but down-regulated in erythroblastic leukemias because the oncogene *c-Kit* is targeted[39] (for other examples, see Table 2). Consequently, it has become evident that miRNAs can affect all the hallmarks of malignant cells: 1) self-sufficiency in growth signals (*let-7* family), 2) insensitivity to antigrowth signals (*miR-17~92* cluster), 3) evasion of apoptosis (*miR-34a*), 4) limitless replicative potential (*miR-372/373* cluster), 5) angiogenesis (*miR-210*), and 6) invasion and metastasis (*miR-10b*).

***Mutations affecting the miRNA-target complex*** Germline and somatic mutations in active pre- or pri-miRNAs may contribute to cancer predisposition and initiation (such as the *miR-15a/16* cluster mutations that occur in rare families with high incidences of both CLL and breast cancer). In the initial report of sequence variations in miRNAs, we reported on two patients diagnosed with CLL, one of whom had a family history of CLL and breast cancer, a C-T homozygous substitution in the *pri-miR-16-1*, and 7 nt in the 3' direction after the end of the pre-miRNA[40]. We found that this substitution was associated with low levels of mature *miR-16* production, revealing a functional impact on the processing of this miRNA. Mutation in a nearly identical location in the 3'-flanking region of *miR-16-1* was described in the New Zealand black (NZB) mouse, a model for human CLL that spontaneously develops the disease when it ages[41]. In another example, scientists screened for genetic variants in 17 selected miRNAs, which were predicted to regulate key breast cancer genes, in 42 patients with familial breast cancer[42]. They identified 7 new variants, 2 in pre-miRNAs (*pre-miR-30c-1* and *pre-miR-21*) and 5 in pri-miRNAs (*pri-miR-17, pri-miR-24-1, pri-miR-125a, pri-miR-191*, and *pri-miR-125b-1*). Interestingly, the variants in *pre-miR-30c-1* and *pri-miR-17* were rare and were only observed in non-carriers of *BRCA1/2* mutations. Since *miR-17* can target *BRCA1* and the described variant affects the processing of *miR-17*[42], miRNA genomic variations can potentially alter the regulation of key breast cancer genes.

Furthermore, polymorphisms in mRNAs targeted by miRNAs influence cancer risk. For example, the *let-7* complementary single nucleotide polymorphism (SNP) sites in the *K-Ras* 3'-UTR that was found to be significantly associated with an increased risk of non–small cell lung carcinoma in moderate smokers[43,44]. Genome-wide bioinformatics analysis has predicted that approximately 64% of transcribed SNPs are target SNPs that can modify (increase or decrease) the binding energy of putative miRNA-mRNA duplexes by more than 90%. To assess whether target SNPs are implicated in breast cancer susceptibility, we conducted a case-control population study and observed that germline occurrence of rs799917-BRCA1 and rs334348–transforming growth factor receptor 1 (TGFR1) significantly varied among populations with different risks of developing breast cancer[45]. Luciferase activity of target SNPs, allelic variants, and protein levels in cancer cell lines with different genotypes showed differential regulation of target genes following overexpression of the two interacting miRNAs (*miR-638* and *miR-628-5p*)[45]. We also reported that a *miR-502*–binding site SNP in the 3'-UTR of the SET8 gene is associated with early age of breast cancer onset[46]. Recently, we found that genetic variants at the *miR-124*–binding site on the cytoskeleton-organizing *IQGAP1* gene confer differential predisposition to breast cancer[47].

## miRNA detection as a diagnostic tool

***Profiling miRNAs—a clinical tool*** The various methods of miRNA profiling have allowed the identification of miRNA signatures associated with diagnosis, staging, progression, prognosis, and treatment response of human cancers. For example, a specific miRNA expression signature consisting of 13 miRNAs in human CLL was reported to link to disease progression from the time of diagnosis to the time of therapy[40]. Similarly, a signature of aberrant expression of 11 miRNAs was found to correlate well with the survival rate of patients with acute myelogenous leukemia (AML)[48]. In another study, researchers identified miRNA expression patterns associated with the incidence, prognosis, and therapeutic outcomes of colon adenocarcinoma by using cancer-specific death as the end point; they reported that miRNAs were differentially expressed in adenomas and adenocarcinomas of the colon and that miRNA expression patterns were associated with survival[49]. They concluded that miRNA expression patterns are systematically altered in colon adenocarcinomas[49]. Metastatic cancer from an unknown primary (CUP) is one of the 10 most frequent cancer diagnoses worldwide and constitutes 3% to 5% of all human malignancies. Patients with CUP present with metastases (i.e., late-stage disease) without an established primary tumor (i.e., a site wherein a therapeutically curative or palliative intervention can be performed). By analyzing 17 poorly differentiated metastatic CUPs with non-diagnostic histological appearance, researchers showed that the miRNA pattern was much better at establishing the correct diagnosis than the mRNA criteria[50]. This result is exciting as it shows that profiling a few hundreds miRNAs has a much greater predictive power for CUP diagnosis than does

profiling tens of thousands of mRNAs for primary coding genes.

***Measurement of plasma miRNAs—a new tool for clinicians*** Measurement of miRNAs in body fluids such as plasma and serum may represent a gold mine of noninvasive analysis of biomarkers in cancers. It has been shown consistently that serum miRNAs remain stable after being subjected to severe conditions that would normally degrade most RNAs, such as boiling, very low or high pH levels, extended storage, and 10 freeze-thaw cycles [51]. Researchers have shown a correlation between circulating miRNA expression levels and response to a given anticancer treatment, as in the case of serum *miR-21* levels that were higher in hormone-refractory prostate cancer patients whose disease was resistant to docetaxel-based chemotherapy than in patients whose disease was chemosensitive [52]. Recently, a high-throughput study generated miRNA signatures from plasma samples collected 12 to 28 months prior to detection of lung cancer and at the time of lung cancer diagnosis [53]. In this study, 21 miRNAs were identified as risk, diagnosis, and prognosis predictors and as being potentially useful in monitoring high-risk disease-free smokers. That study was one of the first to demonstrate that specific pre-disease signatures of miRNA expression in plasma can predict the development of lung cancer prior to diagnosis by conventional techniques and via a noninvasive technique. Furthermore, an independent study proposed that plasma *miR-141* may represent a novel biomarker that complements carcinoembryonic antigen in detecting colon cancer with distant metastasis and that high levels of *miR-141* in plasma were associated with poor prognosis [54].

### Therapeutic potential of miRNAs as drugs or drug targets

Inhibiting RNA by using miRNAs could soon represent a valid option for the treatment of specific patients [55,56]. These patients should have concordant expression between a specific miRNA and the experimentally proven targets. There would be two advantages to using miRNAs: 1) miRNAs are a "natural" product produced in human cells (unlike chemotherapeutic agents or antisense oligonucleotides), and 2) miRNAs target multiple genes from the same pathway and therefore the action occurs at multiple levels in the same pathway (for example, *miR-16* targets both antiapoptotic genes *Bcl-2* and *Mcl-1* [27]). Two strategies for inhibiting RNA expression could be implemented to treat CLL. First, the "sandwich RNA-inhibition strategy" uses multiple agents to focus on a major molecular alteration that is clearly linked to CLL pathogenesis. Given recently published studies showing the relative efficacy of oblimersen sodium in treating relapsed or refractory CLL [57], treatment regimens that combine anti–Bcl-2 oligonucleotides and miRNAs targeting *Bcl-2*, such as *miR-15* and *miR-16*, would be feasible for treating indolent CLL. Second, the "multiplex RNA-inhibition strategy" targets various molecular defects in the same pathway, such as apoptosis. With this strategy, multiple synthetic miRNAs target the overexpressed apoptosis regulators *Bcl-2* (*miR-15* and *miR-16*) and *Mcl-1* (the *miR-29* family) and may have a better chance of consistently and robustly reducing expression of these proteins than does a single-agent therapy. The potential use of miRNAs and/or their antisense inhibitors in cancer treatment has only recently been envisioned, and clinical trials of their use in this manner certainly will be scheduled soon.

## Other ncRNAs Important in Cancers

Despite the leading role of miRNAs as cancer-related ncRNAs in published researches, new categories of untranslated RNAs have recently emerged. ncRNAs are conventionally divided into categories of long and short RNAs. Long ncRNAs include those greater than 200 nt in length; they can reach up to 100 kb [58,59]. Elucidating the functions and characteristics of long ncRNAs is still under way; however, it is already clear that this heterogeneous class displays important regulatory functions, as shown in developmental processes wherein ncRNAs can regulate expression of homeotic genes, oncogenes, and metabolic genes [60]. Despite their smaller size, short ncRNAs are equally important in development, cell biology, and diseases. The discovery of these ncRNAs triggered a general interest in ncRNAs in the scientific community. Other ncRNAs such as large intervening ncRNAs (lincRNAs) and ultraconserved genes (UCGs) were found to be abnormally expressed in cancers and to be involved in tumorigenic mechanisms [61,62]. As the spectrum of ncRNAs is much larger than that of miRNAs (with estimates as high as 1000 000 ncRNA transcripts versus only 10 000 potential miRNAs), the impact on any aspect of basic and translational cancer research will be huge. It was recently discovered that lincRNAs, specifically one called HOTAIR, are involved in cancer metastasis [61]. These lincRNAs have many more nucleotides in their sequences than do short ncRNAs. In fact, long ncRNAs are usually composed of several hundreds nucleotides, as opposed to the 20 or so nucleotides that compose short ncRNAs. The involvement of long non-coding UCGs in cancers is suggested by their frequent location in CAGRs and their aberrant expression in several human cancers. Deregulated UCGs are cancer-specific and have prognostic implications. Similar to miRNAs, UCGs can act as oncogenes or TSGs, and UCG

expression is under the control of miRNAs. Another group of ncRNAs, the short germline-specific P-element induced wimpy testis in Drosophila (PIWI)-associated RNAs, which are involved in the regulation of transposable elements and mRNAs, might have implications for human carcinogenesis, although this connection remains elusive.

## Conclusions

There is no longer any doubt that miRNAs are involved in the regulation of pathways involved in cancer initiation, development, progression, and dissemination. The question of whether miRNAs represent the "dark side" of cancer predisposition is only beginning to be answered by studies in large populations of cancer patients. Despite a few intuitive theories in the 1960s that proposed a regulatory role for RNA in controlling gene expression through base-pair complementarity[63,64], the subsequent discovery of transcription factors quenched further research in this field. As a consequence, for many years RNA has been regarded as the exclusive intermediary molecule between DNA and protein, with the primary role in carrying the genetic information necessary for protein synthesis. Intriguingly, only about 2% of human DNA accounts for protein-coding genes, and the total number of proteins does not vary significantly between different species. On the other hand, the extent of non–protein-coding DNA, regarded for a long time as junk DNA, increases proportionally with developmental complexity[65], and over 90%[66] of the genome is actually transcribed in a developmentally regulated manner to produce ncRNAs that can be intergenic, intronic, or overlapping with protein-coding transcripts[67,68].

miRNAs have been identified as significant new diagnostic and prognostic tools for cancer patients, and miRNA-based cancer therapy is a future option. In addition, ncRNAs display precise tissue- expression patterns[69] and are differentially expressed in pathologic conditions such as cancer and immune and heart diseases [60,62]. A progressive understanding of the implications of ncRNAs for the malignant phenotype represents the essential background needed to achieve the goal of better treatment options for cancer patients.

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