

MicroRNAs in Cancer

Ramiro Garzon,¹ George A. Calin,²
and Carlo M. Croce³

¹Department of Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210; ²Department of Experimental Therapeutics, University of Texas, MD Anderson Cancer Center, Houston, Texas 77020; ³Department of Molecular Virology, Immunology and Medical Genetics and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210; email: carlo.croce@osumc.edu

Annu. Rev. Med. 2009. 60:167–79

The *Annual Review of Medicine* is online at
med.annualreviews.org

This article's doi:
10.1146/annurev.med.59.053006.104707

Copyright © 2009 by Annual Reviews.
All rights reserved

0066-4219/09/0218-0167\$20.00

Key Words

tumors, oncogenes, tumor suppressors, outcome, treatment

Abstract

MicroRNAs (miRNAs) are small, noncoding RNAs with important functions in development, cell differentiation, and regulation of cell cycle and apoptosis. MiRNA expression is deregulated in cancer by a variety of mechanisms including amplification, deletion, mutation, and epigenetic silencing. Several studies have now shown that miRNAs are involved in the initiation and progression of cancer. In this review, we briefly describe miRNA biogenesis and discuss how miRNAs can act as oncogenes and tumor suppressors. We also address the role of miRNAs in the diagnosis, prognosis, and treatment of cancer.

Loss of

heterozygosity: in a heterozygote, the loss of one of the two alleles at one or more loci in a cell lineage or cancer cell population due to chromosome loss, deletion, or mitotic crossing-over

RISC: RNA-induced silencing complex

Epigenetic:

pertaining to control of changes in gene function that do not involve changes in DNA sequences

INTRODUCTION

MicroRNAs (miRNAs) are small, noncoding RNAs ~ 22 nucleotides (nt) in length that regulate gene expression. It has been estimated that miRNAs regulate ~30% of human genes (1). They are involved in the regulation of a variety of biological processes, including cell cycle, differentiation, development, and metabolism (2–5), as well as human diseases, such as diabetes, immuno- or neurodegenerative disorders, and cancer (6–10). Strikingly, half of the known miRNAs are located inside or close to fragile sites and in minimal regions of loss of heterozygosity, minimal regions of amplifications, and common breakpoints associated with cancer (11). For example, the miRNA cluster 17-92 is located at 13q31, a region commonly amplified in lymphomas (12); *miR-143* and *miR-145* are located at 5q33, which is frequently deleted in myelodysplastic syndromes (11); and a rearrangement of *miR-125b-1*, juxtaposed to the immunoglobulin heavy chain locus, was described in a patient with B cell acute lymphocytic leukemia (13).

Following earlier reports (14), several groups, including our own, have systematically analyzed miRNA expression in cancer samples and their corresponding normal tissues (9–10, 14–17). Consequently, miRNA “signatures” were discovered (in both hematological malignancies and solid tumors) that distinguish between tumoral and normal cells, and in some instances are associated with the prognosis and the progression of cancer (9–10, 14–17).

In this review, we briefly describe miRNA biogenesis and discuss how miRNAs can act as oncogenes and tumor suppressors. We also address the issue of miRNA deregulation in the diagnosis, prognosis, and treatment of cancer.

miRNA BIOGENESIS

MiRNA genes are evolutionarily conserved and may be located either within the introns or exons of protein-coding genes (70%) or in intergenic areas (30%) (18). Most of the intronic or exonic miRNAs are oriented in sense with

their host gene, suggesting that they are transcribed in parallel with their host transcript. The second group of miRNAs is transcribed from intergenic regions or gene deserts comprising independent transcription units (18). MiRNAs are preferentially transcribed by polymerase II into long primary transcripts, up to several kilobases (pri-miRNA) that are subsequently processed in the nucleus by the enzyme Drosha to become ~70-nt-long precursor strands (pre-miRNA) (**Figure 1**) (19–20). This precursor is exported by exportin 5 to the cytoplasm (21), where it is bound to the RNase Dicer and to the RNA-induced silencing complex (RISC). RISC is composed of the transactivation-responsive RNA-binding protein (TRBP) and Argonaute 2 (Ago2) (22, 23). Recent studies suggest that first Ago2 cleaves the pre-miRNA 12 nt from its 3' end (forming Ago2-cleaved precursor miRNA) and then the Dicer cleaves the Ago2-cleaved precursor miRNA into a mature 22-nt miRNA duplex (24). While the active or mature strand is retained in RISC, the passenger strand is removed and degraded (1, 22–24). For the most part, the mature 22-nt strand recognizes complementary sequences in the 3' untranslated region of target mRNAs—particularly the seed sequence at the 5' end (2–8 nt)—and guides the miRNA-RISC complex to repress gene expression by inhibiting translation and inducing mRNA degradation (**Figure 1**) (1, 22–24). During miRNA biogenesis, miRNAs are subject to intense transcriptional and post-transcriptional regulation, and the elucidation of these mechanisms has improved our understanding of miRNA deregulation in disease.

miRNAs AS TUMOR SUPPRESSORS

Like a protein-coding gene, a miRNA can act as a tumor suppressor when its function loss can initiate or contribute to the malignant transformation of a normal cell. The loss of function of a miRNA could be due to several mechanisms, including genomic deletion, mutation, epigenetic silencing, and/or miRNA processing alterations (14–15, 25–26). For example, the

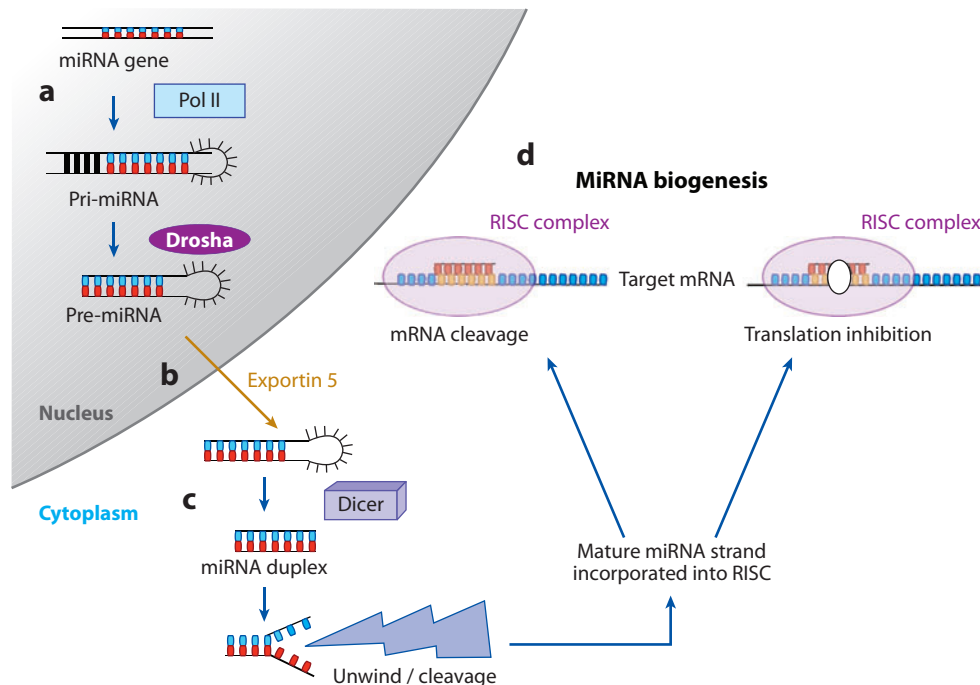


Figure 1

MicroRNA biogenesis. (a) MiRNAs are transcribed by RNA polymerase II (pol II) into long primary miRNA transcripts of variable size (pri-miRNA), which are recognized and cleaved in the nucleus by the RNase III enzyme Drosha, resulting in a hairpin precursor form called pre-miRNA. (b) Pre-miRNA is exported from the nucleus to the cytoplasm by exportin 5 and is further processed by another RNase enzyme called Dicer (c), which produces a transient 19–24-nt duplex. Only one strand of the miRNA duplex (mature miRNA) is incorporated into a large protein complex called RISC (RNA-induced silencing complex). (d) The mature miRNA leads RISC to cleave the mRNA or induce translational repression, depending on the degree of complementarity between the miRNA and its target. Reproduced with permission from Reference 24a.

most frequently observed chromosomal abnormality in chronic lymphocytic leukemia (CLL) is the hemizygous and/or homozygous deletion of the 13q14.3 region, which occurs in >50% of cases and is associated with indolent disease (27–28). All attempts to identify any tumor suppressor gene located in this chromosomal region that could explain CLL pathogenesis failed until recently, when a cluster of miRNAs, namely *miR-15a* and *miR-16-1*, was discovered in the 13q14.2 region, mapping to the 30-kb deleted region between exons 2 and 5 of the *LEU2* gene (14). In a subset of CLLs, the *miR-15a/16-1* cluster is observed to be deleted or downregulated when compared to normal CD5⁺ lymphocytes from healthy

donors (Table 1) (14). In addition to deletion, a mutation responsible for silencing the cluster's expression has been described, associated with the loss of the normal allele, in the leukemic cells of two CLL patients (15). A 3' point mutation adjacent to the *miR-16-1* region, which results in reduced expression of this miRNA, was found in the New Zealand Black mouse model, a strain characterized by naturally occurring late-onset CLL (29). Evidence of a tumor suppressor role for this cluster was provided by the discovery that the antiapoptotic gene *BCL-2*, which is widely overexpressed in CLL by unknown mechanisms, is a bona fide target of the *miR-15a/miR-16-1* cluster (30). Therefore, low levels of *miR-15a/miR-16-1* due

CLL: chronic lymphocytic leukemia

Table 1 MiRNAs with experimental data supporting a tumor suppressor or oncogene function in cancer

MicroRNA	Expression in patients	Confirmed targets	Experimental data	Function	References
<i>miR-15a</i> <i>miR-16-1</i>	downregulated in CLL ^a	Bcl-2, Wt-1	induce apoptosis and decrease tumorigenicity	TS ^a	14, 30–32
<i>let-7</i> (<i>a</i> , <i>b</i> , <i>c</i> , <i>d</i>)	downregulated in lung and breast cancer	RAS, c-myc, HMGA2	induce apoptosis	TS	16–17, 33–36
<i>miR-29</i> (<i>a</i> , <i>b</i> , <i>c</i>)	downregulated in CLL, AML ^a (11q23), lung and breast cancers, and cholangiocarcinoma	TCL-1, MCL1, DNMT3s	induce apoptosis and decrease tumorigenicity	TS	15–17, 38–41
<i>miR-34a-b-c</i>	downregulated in pancreatic, colon, and breast cancers	CDK4, CDK6, cyclinE2, E2F3	induce apoptosis	TS	69–72
<i>miR-155</i>	upregulated in CLL, DLBCL, ^a FLT3-ITD ^a AML, BL, ^a and lung and breast cancers	c-maf	induces lymphoproliferation, pre-B lymphoma/leukemia in mice	OG ^a	10, 16–17, 38, 43–49
<i>miR-17~92</i> cluster	upregulated in lymphomas and in breast, lung, colon, stomach, and pancreas cancers	E2F1, Bim, PTEN	cooperates with c-myc to induce lymphoma in mice, transgenic miR-17-92 develop lymphoproliferative disorder	OG	10, 60, 57–67
<i>miR-21</i>	upregulated in breast, colon, pancreas, lung, prostate, liver, and stomach cancer; AML(11q23); CLL; and glioblastoma	PTEN, PDCD4, TPM1	induces apoptosis and decreases tumorigenicity	OG	10, 15, 38, 46, 50–55
<i>miR-372/</i> <i>miR-373</i>	upregulated in testicular tumors	LATS2	promote tumorigenesis in cooperation with RAS	OG	68

^aAbbreviations: CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; DLBCL, diffuse large B cell lymphoma; FLT3-ITD, FMS-like tyrosine kinase 3 in tandem duplication mutations; BL, Burkitt lymphoma; TS, tumor suppressor; OG, oncogene.

to genomic deletion (or, less frequently, mutations) may unblock BCL-2 protein expression in a subset of CLL patients. Consistent with this hypothesis, *miR-15a/miR-16-1* expression levels are inversely correlated to BCL-2 expression in CLL patients (30).

Furthermore, ectopic expression of *miR-16-1* negatively regulates cell growth and cell cycle progression and induces apoptosis in several human cancer cell lines (30–31) and in a leukemic xenograft model (32).

In a similar vein, the *let-7* family of miRNAs is downregulated in many tumors, including lung and breast cancer (16–17, 33). Many of the *let-7* family members are located in fragile genomic areas associated with lung, breast, and cervical cancer (11). Finally, *let-7* family members functionally inhibit the mRNAs of well-characterized oncogenes, such as the

Ras family (33–34), *HMGA2* (35), and *c-myc* (36), and induce apoptosis and cell cycle arrest when overexpressed in lung and colon cancer and in Burkitt lymphoma cell lines (**Table 1**) (34–36).

The *miR-29* family comprises three isoforms arranged in two clusters: *miR-29b-1/miR-29a* in chromosome 7q32 and *mir-29b-2/miR-29c* in chromosome 1q23. Interestingly, chromosome 7q32 is a frequent region of deletion in myelodysplasia and therapy-related acute myeloid leukemia (AML) (37). In fact, *miR-29* family members have been shown to be downregulated in CLL, lung cancer, invasive breast cancer, AML, and cholangiocarcinoma (**Table 1**) (15–17, 38–39). The enforced expression of *miR-29b* induced apoptosis in cholangiocarcinoma and lung cancer cell lines and reduced tumorigenicity in a xenograft

model of lung cancer (39, 40). These profound tumor suppressor effects can be explained in part by the direct targeting of the antiapoptotic protein MCL-1 and the oncogene *TCL-1* by the *miR-29* family (39, 41).

Many other miRNAs are believed to act as tumor suppressors, although the evidence supporting those claims is merely correlative. Substantial experimental data are lacking, and miRNA knockout mice that develop or are predisposed to cancer have not been yet reported. It is noteworthy that most of the miRNAs with a clear tumor suppressor role (e.g., *miR-15a/16-1*, *miR-29s*, and *let-7*) have more than one genomic location, and although they are transcribed from different precursors, the mature miRNA is identical. The different loci could be differentially regulated; for example, in HeLa cells the mature *miR-29b* is preferentially transcribed from the *miR-29b-1/miR-29a* locus in chromosome 7q32, whereas the other locus, *miR-29b-2/miR-29c* in chromosome 1q23, is silenced (42). The presence of more than one genomic copy of the miRNA could be an evolutionarily conserved mechanism to preserve function of an important miRNA if one allele is deleted or silenced.

miRNAs AS ONCOGENES

The list of miRNAs that function as oncogenes is short, but the evidence for their role in cancer is very strong (Table 1). *MIR-155* was one of the first described (43–44). It is embedded in a host noncoding RNA named the B cell integration cluster (*BIC*) and is located in chromosome 21q23 (45). A previous study showed the ability of *BIC* to cooperate with *c-myc* in oncogenesis. The coexpression of *c-myc* and *BIC*, either singly or pairwise, in cultured chicken embryo fibroblasts using replication-competent retrovirus vectors caused growth enhancement of cells (45). Several groups have shown that *miR-155* is highly expressed in pediatric Burkitt lymphoma (43), Hodgkin disease (44), primary mediastinal non-Hodgkin lymphoma (44), CLL (15), AML (38), lung cancer (10, 16), and breast cancer (10, 17).

Little is known, however, about the regulation of *miR-155/BIC* and the mechanism of its overexpression in cancer. In AML, this miRNA is positively correlated with high white counts and the presence of FLT3 in tandem duplication (ITD) mutations (38, 46). Further experiments confirmed that *miR-155* expression is independent of FLT3-ITD, since blocking FLT3-ITD signaling in human leukemic cells with a potent FLT3 inhibitor did not affect *miR-155* levels (46). A role for this miRNA in early leukemogenesis was proven in a transgenic mouse model with a B cell-targeted overexpression of *miR-155*, which underwent a polyclonal preleukemic pre-B cell proliferation followed by full-blown B cell malignancy (Table 1) (47). More recently, two knockout mouse models have demonstrated the critical role of *miR-155* in immunity: *BIC/miR-155*^{-/-} mice have defective dendritic cell functions, impaired cytokine secretion, and Th cells intrinsically biased toward Th2 differentiation (48–49).

There is also strong evidence that *miR-21* functions as an oncogene. First, this miRNA is upregulated in a wide variety of hematological malignancies and solid tumors, including AML (38, 46), CLL (15), glioblastoma (50), and cancers of the pancreas, prostate, stomach, colon, lung, breast (10), and liver (51) (Table 1). Second, overexpressing *miR-21* in glioblastoma cells blocks apoptosis (52), whereas silencing its expression in cultured liver glioblastoma and breast cancer cells using antisense oligonucleotides inhibits cell growth, triggers activation of caspases, and increases apoptotic cell death (51–53) by targeting tumor suppressor genes such as *PTEN* (phosphatase and tensin homolog) (54), *PDCD4* (programmed cell death 4) (53), and *TPM1* (tropomyosin 1) (55).

The *miR-17-92* cluster, which comprises six miRNAs (*miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1*, and *miR-92-1*), is located within 800 base pairs in the noncoding gene C13orf25 at 13q31.3. This region is frequently amplified in follicular lymphoma and diffuse large B cell lymphoma (12). In addition to being key players in lung development (56) and in regulation

of the immune and hematopoietic systems (57–58), members of the *miR-17-92* cluster are highly expressed in a variety of solid tumors and hematological malignancies, including cancers of the breast, colon, lung, pancreas, prostate, and stomach as well as lymphomas (**Table 1**) (10, 59, reviewed in Reference 60). These miRNAs promote proliferation, inhibit apoptosis, induce tumor angiogenesis, and cooperate with *c-myc* to cause lymphoma in mice (60–62). Interestingly, the *miR-17-92* cluster is transactivated by *c-myc*, an oncogene that is frequently activated in cancer (63). Two recent papers using gain- and loss-of-function strategies reported that this cluster is essential for B cell proliferation and that a modest overexpression in B cells induces a lymphoproliferative disorder in mice (57–58). The effects of this cluster's expression on cell cycle and proliferation are partly due to its regulation of E2F transcription factors, which are critical regulators of the cell cycle (63). Genes in the E2F family, particularly *E2F1*, *E2F2*, and *E2F3*, activate multiple genes resulting in cell cycle progression from G1 to S phase (64). Conversely, both *E2F1* and *E2F3* can activate the *miR-17-92* cluster, establishing a regulatory loop (63). Critical also to the antiapoptotic effects of this cluster is the down-modulation of two validated targets: the antiapoptotic protein Bim and the tumor suppressors *PTEN* and *p21* (57–58, 65). The current theory is that like *miR-155*, this cluster induces lymphoid proliferation that predisposes to secondary genetic abnormalities that will ultimately become a full-blown malignancy. Intriguingly, isolated *miR-17-5p* downregulation has been reported in breast cancer cell lines (66). Restoring its expression decreased breast cancer cell proliferation by inhibiting translation of the *AIB1* (amplified in breast cancer) gene. Deletion of the *miR-17-92* genomic locus has been described in 16.5% of ovarian cancers, 21.9% of breast cancers, and 20% of melanomas (60, 66).

These observations raise the question whether there is a fine post-transcriptional mechanism that regulates the expression of individual miRNAs in this cluster. In addition, these data support a tumor suppressor role for

miR-17-5p, which seems to contradict extensive data consistently showing upregulation of this cluster in cancer (10). This dual role, oncogene and tumor suppressor, has also been described in protein-coding genes involved in the pathogenesis of cancer, such as *TP53* (67). It is possible that a miRNA can act either as an oncogene or as a tumor suppressor depending on the tissue and its transcriptome, including the miRNA targets expressed in that particular tissue.

Using a novel miRNA expression vector library containing the majority of cloned human miRNAs, Voorhoeve et al. performed a screening for miRNAs that cooperate with oncogenes in cellular transformation (68). They identified two miRNAs, *miR-372* and *miR-373*, which induced proliferation and tumorigenesis of primary human cells in cooperation with *Ras* by neutralizing wild-type gene *TP53* through direct inhibition of the expression of the tumor suppressor gene *LATS2*. They found that this mechanism participates in the oncogenesis of human testicular germ cell tumors, allowing oncogenic growth by targeting the wild-type *TP53* pathway.

EPIGENETIC REGULATION OF miRNAs

As described above, miRNAs deregulation in cancer could result in part from genomic deletion, mutation, or amplification (14–15, 25–26). In addition, epigenetic mechanisms that regulate miRNA expression in cancer have been described (69–71). A cluster of papers in *Nature* and *Molecular Cell* revealed that a family of miRNAs, *miR-34a*, *-b*, and *-c*, are induced directly by *TP53* and suggested that some of *TP53*'s effects could be mediated through transcriptional activation of miRNAs (69–71). Using different models, the authors compared miRNA expression in cells with high or low *TP53* expression and found that *miR-34* expression is increased in cells with high *TP53* levels. Chromatin immunoprecipitation experiments revealed that *TP53* binds to the *miR-34s*' promoters. Restoring *miR-34* levels in both primary and tumor cell lines induced

cell cycle arrest by targeting a gene program involved in cell cycle progression (69–71).

We have discussed how *c-myc* transactivates miRNAs, in particular the *miR-17-92* cluster (63). Recent work also suggests that *c-myc* negatively regulates transcription of tumor suppressor miRNAs, such as *let-7* (*let-7a-1*, *let-7f-1*, *let-7d*, *let-7c* and *let-7g*) and *miR-29* family members (*-a*, *-b*, and *-c*) (72). Chromatin immunoprecipitation experiments showed that *c-myc* binds to conserved sequences of the miRNA promoter that it represses. Functionally *c-myc*-induced repression of miRNAs contributes to lymphomagenesis, since the restoration of the silenced miRNAs decreases the tumorigenic potential of the lymphoma cells (72). Taken together, current data suggest that miRNAs play important roles in the *c-myc*, *E2F*, and *TP53* oncogenic pathways through the coordinated regulation of multiple transcripts (63, 65, 69–72).

Another important epigenetic mechanism of miRNA regulation is miRNA expression silencing by promoter DNA hypermethylation. Saito et al. first reported that *miR-127* is silenced by promoter hypermethylation in bladder cancer cell lines and patients, and its expression could be restored by using hypomethylating agents (25). Furthermore, the authors showed that this miRNA targets the oncogene *BCL-6*, suggesting that hypomethylating agents can activate expression of miRNAs (25). Other groups have described different miRNAs that are silenced by methylation and that when re-expressed behave as tumor suppressors (73–74). MiRNAs not only are regulated by DNA methylation but also modulate DNA methylation in cancer by interfering with the DNA methylation machinery (38). Our group has reported that the *miR-29* family, which includes targets *DNMT3A* and *-3B*, induces global DNA hypomethylation and tumor suppressor gene re-expression in lung cancer (38). Interestingly, *miR-29s* are down-regulated in lung cancer patients, and an inverse correlation was found between *miR-29b* and *DNMT3B* expression, suggesting that the downregulation of this miRNA may contribute to increased DNMT3 levels, as well as hyper-

methylation and silencing of tumor suppressor genes in lung cancer (16, 40).

miRNA PROFILING TO IMPROVE DIAGNOSIS AND OUTCOME PREDICTION

It has been shown that miRNAs can differentiate between tissue types with high accuracy (9). The use of miRNA profiling outperformed cDNA microarrays in the classification of tumors of unknown primary. Indeed, Lu et al. (9) found that only a few miRNAs were needed to accurately predict the tumor tissue of origin. MiRNA profiling could represent an invaluable tool to classify tumors that represent diagnostic challenges. The discovery of distinctive miRNA signatures will likely improve the molecular classification of cancer.

Several studies have shown that miRNA expression is predictive of outcome in solid tumors and hematological malignancies (Table 2) (15, 38, 75–78). A signature of nine miRNAs (including high levels of *miR-155*, *miR-221*, and *miR-222* and low levels of *miR-29c*) was clearly associated with time to progression in CLL (15). In lung cancer, low levels of *let-7a* were associated with short survival after surgery in two independent studies (16, 75). A subgroup of six miRNAs was able to distinguish long-term pancreatic cancer survivors with node-positive disease from those who died within 24 months (76). High expression of *miR-196a-2* was found to predict poor survival [median, 14.3 months (95% confidence interval, 12.4–16.2) versus 26.5 months (95% confidence interval, 23.4–29.6); $p = 0.009$] (76). When miRNA microarray expression profiling of tumors and paired nontumorous tissues was performed in 197 colon cancer patients to identify miRNA expression patterns associated with outcome, high levels of *miR-21* were associated with short overall survival, independent of other factors (77).

Two studies have shown that miRNAs (independent of other factors) are associated

Table 2 MicroRNAs associated with outcome in cancer

MicroRNA	Disease	Expression in poor outcome	Variable	References
<i>let-7a</i>	non–small cell lung cancer	low	OS ^a	16, 75
<i>miR-21</i>	colon adenocarcinoma	high	OS, DFS ^a	77
<i>miR-21</i>	CLL ^a	high	time to progression	15
<i>miR-155</i>	CLL	high	time to progression	15
<i>miR-221/222</i>	CLL	high	time to progression	15
<i>miR-146</i>	CLL	high	time to progression	15
<i>miR-29-c</i>	CLL	low	time to progression	15
<i>miR-196-a</i>	pancreas adenocarcinoma	high	OS	76
<i>miR-191</i>	AML ^a	high	OS, DFS	38
<i>miR-199a</i>	AML	high	OS, DFS	38
<i>miR-181a/b</i>	CN-AML ^a (<60 years)	low	DFS	78
<i>miR-181a</i>	CLL	high	time to progression	15

^aAbbreviations: OS, overall survival; DFS, disease-free survival; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; CN-AML, cytogenetically normal AML.

with overall and disease-free survival in AML (Table 2) (38, 78). Garzon et al. analyzed 120 newly diagnosed AML patients with intermediate and poor cytogenetics groups and found five upregulated miRNAs associated with poor outcome (38). All the identified miRNAs, *miR-199a*, *miR-199b*, *miR-191*, *miR-20*, and *miR-25*, when overexpressed, adversely affected overall survival. The authors confirmed the results for *miR-191* and *miR-199* using an independent set of 60 patients and a different profiling method. A second study reported a miRNA signature associated with overall and disease-free survival in a cohort of high-risk cytogenetically normal AML patients (78). The signature was characterized by upregulation of five probes that corresponded to *miR-181a* and *miR-181b* family members and downregulation of seven probes including *miR-124*, *miR-204*, *miR-194*, *miR-320*, and *219-5p*. Expression of the five probes corresponding to *miR-181a/b* was inversely associated with the risk of event (i.e., death or relapse), whereas the other probes were positively correlated with the risk of event. These results were validated using an independent cohort of cytogenetically normal AML patients, and the signature remained significant after adjusting for other variables (78).

The different signatures between the two studies could be explained by differences in age [median 60.3 years (range 18–86) versus median 45 years (range 19–59)], cytogenetics group frequencies (the first study included intermediate- and poor-risk cytogenetics groups, whereas the second study included only cytogenetically normal patients), and treatment (34, 78). In summary, miRNA profiling has been proven a valuable tool to predict outcome. However, further studies will be needed to test whether miRNAs could be used to better stratify patients for treatment.

miRNAs IN TUMOR INVASION AND METASTASIS

Very few studies have addressed the role of miRNAs in tumor invasion and metastasis. Ma et al. found that *miR-10b* was upregulated in metastatic breast cancer cells with respect to the primary tumors (79). They reported that enforced expression of *miR-10b* in nonmetastatic breast tumor cells positively regulated cell migration and invasion and that the level of *miR-10b* expression in primary breast cancer tissues correlated with clinical progression. However, only 23 patient samples were analyzed,

and these results warrant further independent validation. Using a different approach, Huang et al. analyzed a nonmetastatic breast cancer cell line migration after transduction with a miRNA expression library (80). The authors identified two miRNAs, *miR-373* and *miR-520c*, that stimulated cancer cell migration and invasion in vitro and in vivo by blocking the adhesion molecule CD44. A significant upregulation of *miR-373* and negative correlation with CD44 expression was found in breast cancer patients with metastasis.

miRNAs AS THERAPEUTIC TARGETS

There are several reasons to pursue a miRNA-based therapeutic approach. First, a single miRNA can have many targets that are involved in different oncogenic pathways. For example, *miR-29b* targets MCL-1 (apoptosis) and DNMT3A and -3B (methylation) (39, 40); *miR-181* targets BCL-2 (apoptosis) (81), TCL-1 (AKT pathway) (41), and CD69 (adhesion) (81); and *miR-17-92* targets the E2F family (cell cycle), Bim (apoptosis), and angiogenesis (61). Therefore, modulating the level of a single miRNA could eventually affect many pathways at the same time. Second, since a small group of miRNAs, including *miR-155*, *let-7a*, *miR-21*, and the *miR-17-92* cluster, are consistently deregulated in

a wide variety of hematological malignancies and solid tumors (10), developing strategies to silence or re-express these miRNAs will likely affect several groups of patients. Third, as a proof of principle, preliminary data indicate that using cholesterol-modified antisense oligonucleotides to the mature miRNAs (named antagomirs) is an effective approach to silence miRNA expression in mice (82). This could be a valuable approach to silence miRNAs upregulated in cancer, such as *miR-155* or *miR-21*. However, the use of cholesterol-based oligonucleotides could be too toxic for humans. Elmén et al. recently reported that the simple systemic delivery of an unconjugated locked-nucleic acid modified oligonucleotide (LNA-antimiR) effectively antagonized the liver-expressed *miR-122* in non-human primates (83). Using three intravenous doses of 10 mg/kg in African green monkeys, the authors observed effective depletion of the *miR-122* without any evidence of LNA-associated toxicities or histopathological changes in the animals. Further research is needed to determine the best formulation. In addition, precise delivery to the cancer cell is needed to avoid unwanted miRNA effects that could result from targeting important genes in other healthy tissues. Promising miRNA formulations should be further evaluated by detailed pharmacokinetics and pharmacodynamics studies in animal models.

Antagomir: member of a novel class of chemically engineered oligonucleotides, which are efficient and specific silencers of endogenous miRNAs. They are synthesized from a hydroxyprolinol-linked cholesterol solid support and 2'-OMe phosphoramidites

Locked-nucleic acid modified oligonucleotide: bicyclic nucleotide analogue that significantly increases the melting temperature of hybrids with miRNAs

SUMMARY POINTS

1. Half of the known miRNAs are located inside or close to fragile sites and in minimal regions of loss of heterozygosity, minimal regions of amplification, and common breakpoints associated with cancer.
2. The loss of function of a miRNA could be due to several mechanisms, including genomic deletion, mutation, epigenetic silencing, and/or miRNA processing alterations.
3. MiRNAs can act as oncogenes or tumor suppressors depending on the tissue and the expression of their targets.
4. MiRNAs with a tumor suppressor function frequently have more than one genomic locus. This could be a natural defense against cancer, preserving tumor suppressor miRNA levels in the event of loss or mutation of one locus.

5. A small group of miRNAs, including *miR-155*, *let-7a*, *miR-21*, and the *miR-17-92* cluster, are aberrantly expressed in a wide variety of hematological malignancies and solid tumors. Developing a strategy to silence or restore the expression of these oncogenic miRNAs would have an impact on multiple groups of cancer patients.
6. Because miRNAs can target many genes, modulating the level of a single miRNA could eventually affect many pathways at the same time.

FUTURE ISSUES

1. Further studies are needed to test whether miRNAs could be used to better stratify cancer patients for treatment.
2. Detailed pharmacokinetics and pharmacodynamics studies are required to assess what dose of oligonucleotides should be used.
3. Animal models of loss or gain of allele function are needed to establish the role of miRNAs in cancer.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to all investigators whose research could not be cited owing to space limitations.

LITERATURE CITED

1. Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116:281–97
2. Carleton M, Cleary MA, Linsley PS. 2007. MicroRNAs and cell cycle regulation. *Cell Cycle* 6:2127–32
3. Harfe BD. 2005. MicroRNAs in vertebrate development. *Curr. Opin. Genet. Dev.* 15:410–15
4. Lau NC, Lim LP, Weinstein EG, et al. 2001. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294:858–62
5. Boehm M, Slack FJ. 2005. MicroRNA control of lifespan and metabolism. *Cell Cycle* 5:837–40
6. Poy MN, Eliasson L, Krutzfeldt J, et al. 2004. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432:226–30
7. Landthaler M, Yalcin A, Tuschl T. 2004. The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. *Curr. Biol.* 14:2162–67
8. Jin P, Alisch RS, Warren ST. 2004. RNA and microRNAs in fragile X mental retardation. *Nat. Cell Biol.* 6:1048–53
9. Lu J, Getz G, Miska EA, et al. 2005. MicroRNA expression profiles classify human cancers. *Nature* 435:834–38
10. Volinia S, Calin G, Liu CG, et al. 2006. A microRNA expression signature in human solid tumors defines cancer targets. *Proc. Natl. Acad. Sci. USA* 103:2257–61
11. Calin GA, Sevignani C, Dumitru CD, et al. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. USA* 101:2999–3004

12. Ota A, Tagawa H, Karnan S, et al. 2004. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer Res.* 64:3087-95
13. Sonoki T, Iwana E, Mitsuya H, et al. 2005. Insertion of microRNA-125b-1, a human homologue of lin-4, into a rearranged immunoglobulin heavy chain gene locus in a patient with precursor B-cell acute lymphoblastic leukemia. *Leukemia* 19:2009-10
14. Calin GA, Dumitru C, Shimizu M, et al. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA* 99:15524-29
15. Calin GA, Ferracin M, Cimmino A, et al. 2005. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N. Engl. J. Med.* 353:1793-801
16. Yanaihara N, Caplen N, Bowman E, et al. 2006. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9:189-98
17. Iorio MV, Ferracin M, Liu CG, et al. 2005. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 65:7065-70
18. Rodriguez A, Griffiths-Jones S, Ashurst JL, et al. 2004. Identification of mammalian microRNA host genes and transcription units. *Genome Res.* 14:1902-10
19. Lee Y, Kim M, Han J, et al. 2004. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* 23:4051-60
20. Lee Y, Ahn C, Han J, et al. 2003. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 425:415-19
21. Bohnsack MT, Czaplinski K, Gorlich D. 2004. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of premiRNAs. *RNA* 10:185-91
22. Hammond S, Bernstein E, Beach D, et al. 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404:293-329
23. Thimmaiah P, Chendrimada GR, Kumaraswamy E, et al. 2005. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* 436:740-44
24. Diederichs S, Haber DA. 2007. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. *Cell* 131:1097-108
- 24a. Garzon R, Fabbri M, Cimmino A, et al. 2006. MicroRNA expression and function in cancer. *Trends Mol. Med.* 12:580-87
25. Saito Y, Liang G, Egger G, et al. 2006. Specific activation of microRNAs-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 9:435-43
26. Nakamura T, Canaani E, Croce CM. 2007. Oncogenic All1 fusion proteins target Drosha-mediated microRNA processing. *Proc. Natl. Acad. Sci. USA* 104:10980-85
27. Bullrich F, Fujii H, Calin G, et al. 2001. Characterization of the 13q14 tumor suppressor locus in CLL: identification of ALT1, an alternative splice variant of the LEU2 gene. *Cancer Res.* 61:6640-48
28. Doehner H, Stilgenbauer S, Benner A, et al. 2000. Genomic aberrations and survival in chronic lymphocytic leukemia. *N. Engl. J. Med.* 343:1910-16
29. Raveche ES, Salerno E, Scaglione BJ, et al. 2007. Abnormal microRNA-16 locus with synteny to human 13q14 linked to CLL in NZB mice. *Blood* 109:5079-86
30. Cimmino A, Calin GA, Fabbri M, et al. 2006. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl. Acad. Sci. USA* 102:13944-49
31. Linsley PS, Schelter J, Burchard J, et al. 2007. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol. Cell Biol.* 27:2240-52
32. Calin GA, Cimmino A, Fabbri M, et al. 2008. MiR-15a and miR-16-1 cluster functions in human leukemia. *Proc. Natl. Acad. Sci. USA* 105:5166-71
33. Johnson SM, Grosshans H, Shingara J, et al. 2005. RAS is regulated by the let-7 microRNA family. *Cell* 120:635-47
34. Akao Y, Nakagawa Y, Naoe T. 2006. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Biol. Pharm. Bull.* 29:903-6
35. Lee YS, Dutta A. 2007. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev.* 21:1025-30
36. Sampson VB, Rong NH, Han J, et al. 2007. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res.* 67:9762-70

37. Pedersen-Bjergaard J, Pedersen M, Roulston D, et al. 1995. Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. *Blood* 86:3542–52
38. Garzon R, Volinia S, Liu CG, et al. 2008. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* 111:3183–89
39. Mott JL, Kobayashi S, Bronk SF, et al. 2007. Mir-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* 26:6133–40
40. Fabbri M, Garzon R, Cimmino A, et al. 2007. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc. Natl. Acad. Sci. USA* 104:15805–10
41. Pekarsky Y, Santanam U, Cimmino A, et al. 2006. TCL1 expression in CLL is regulated by miR-29 and miR-181. *Cancer Res.* 66:11590–93
42. Hwang HW, Wentzel EA, Mendell JA. 2007. A hexanucleotide element directs microRNA nuclear import. *Science* 315:97–100
43. Metzler M, Wilda M, Busch K, et al. 2004. High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. *Genes Chrom. Cancer* 39:167–69
44. Kluiver J, Poppema S, de Jong D, et al. 2005. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J. Pathol.* 207:243–49
45. Tam W, Hughes SH, Hayward WS, Besmer P. 2002. Avian bic, a gene isolated from a common retroviral site in avian leukosis virus-induced lymphomas that encodes a noncoding RNA, cooperates with c-myc in lymphomagenesis and erythroleukemogenesis. *J. Virol.* 76:4275–86
46. Garzon R, Garofalo M, Martelli MP, et al. 2008. Distinctive miRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. *Proc. Natl. Acad. Sci. USA* 105:3945–50
47. Costinean S, Zanesi N, Pekarsky Y, et al. 2006. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in Eμ-miR155 transgenic mice. *Proc. Natl. Acad. Sci. USA* 103:7024–29
48. Thai TH, Dinis P, Casola S, et al. 2007. Regulation of the germinal center response by microRNA-155. *Science* 316:604–8
49. Rodriguez A, Vigorito E, Clare S, et al. 2007. Requirement of BIC/microRNA-155 for normal immune function. *Science* 316:608–11
50. Ciafre SA, Galardi S, Mangiola A, et al. 2005. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem. Biophys. Res. Commun.* 334:1351–58
51. Meng F, Henson RH, Wehbe-Janek H, et al. 2007. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133:647–58
52. Chan JA, Krichevsky AM, Kosik KS. 2005. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 65:6029–33
53. Frankel LB, Christoffersen NR, Jacobsen A, et al. 2008. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J. Biol. Chem.* 283:1026–33
54. Meng F, Henson R, Wehbe-Janek H, et al. 2007. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 133:647–58
55. Zhu S, Si ML, Wu H, Mo YY. 2007. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J. Biol. Chem.* 282:14328–36
56. Lu, JM, Thomson HY, Wong SM, et al. 2007. Transgenic overexpression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Dev. Biol.* 310:442–53
57. Ventura AG, Young MM, Winslow L, et al. 2008. Targeted deletion reveals essential and overlapping functions of the miR-17~92 family of miRNA clusters. *Cell* 132:875–86
58. Xiao C, Srinivasan L, Calao DP, et al. 2008. Lymphoproliferative disease and autoimmunity in mice with elevated miR-17-92 expression in lymphocytes. *Nat. Immunol.* 9:405–14
59. Venturini L, Battmer K, Castoldi M, et al. 2007. Expression of the *miR-17-92* polycistron in chronic myeloid leukemia (CML) CD34⁺ cells. *Blood* 109:4399–405
60. Mendell JT. 2008. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* 133:217–22. doi:10.1016/j.cell.2008.04.001
61. Dews M, Homayouni A, Yu D, et al. 2006. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. *Nat. Genet.* 38:1060–65

62. He L, Thomson M, Hemann MT, et al. 2005. A microRNA polycistron as a potential human oncogene. *Nature* 435:828–33
63. O'Donnell KA, Wentzel EA, Zeller KI, et al. 2005. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 435:839–43
64. Leone G, DeGregori J, Sears R, et al. 1997. Myc and Ras collaborate in inducing accumulation of active cyclin E/Cdk2 and E2F. *Nature* 387:422–26
65. Petrocca R, Visone MR, Onelli MH, et al. 2008. E2F1-regulated microRNAs impair TGF β -dependent cell cycle arrest and apoptosis in gastric cancer. *Cancer Cell* 13:272–86
66. Hossain M, Kuo T, Saunders GF. 2006. miR-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol. Cell. Biol.* 26:8191–201
67. Lane DP, Benchimol S. 1990. p53: oncogene or antioncogene? *Genes Dev.* 4:1–8
68. Voorhoeve PM, le Sage C, Schrier M, et al. 2007. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* 124:1169–81
69. He L, He X, Lim L, et al. 2007. A microRNA component of the p53 tumour suppressor network. *Nature* 447:1130–34
70. Raver-Shapira N, Marciano E, Meiri E, et al. 2007. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol. Cell* 26:731–43
71. Chang TC, Wentzel EA, Kent OA, et al. 2007. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol. Cell* 26:745–52
72. Chang TS, Yu D, Lee YS, et al. 2007. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat. Genet.* 40:43–50
73. Lujambio A, Ropero S, Ballestar E, et al. 2007. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res.* 67:1424–29
74. Lu L, Katsaros D, Rigault de la Longrais DA, et al. 2007. Hypermethylation of let-7a-3 in epithelial ovarian cancer is associated with low insulin-like growth factor-II expression and favorable prognosis. *Cancer Res.* 67:10117–22
75. Takamizawa J, Konishi H, Yanagisawa K, et al. 2004. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res.* 64:3753–56
76. Bloomston M, Frankel WL, Petrocca F, et al. 2007. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 297:1901–8
77. Schetter AJ, Leung SY, Sohn JJ, et al. 2008. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 299(8):425–36
78. Marcucci G, Radmacher MD, Maharry K, et al. 2008. MicroRNA expression in cytogenetically normal acute myeloid leukemia. *N. Engl. J. Med.* 358:1919–28
79. Ma L, Teruya-Feldstein J, Weinberg RA. 2007. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449:682–88
80. Huang H, Gumireddy K, Schrier M, et al. 2008. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat. Cell Biol.* 10:202–10
81. Neilson JR, Zheng GXY, Burge CB, et al. 2007. Dynamic regulation of microRNAs expression in ordered stages of cellular development. *Genes Dev.* 21:578–89
82. Krutzfeldt J, Rajewsky N, Braich R, et al. 2005. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 438:685–89
83. Elmén J, Lindow M, Schütz S, et al. 2008. LNA-mediated microRNA silencing in nonhuman primates. *Nature* 452:896–99



Contents

Transcatheter Valve Repair and Replacement <i>Susheel Kodali and Allan Schwartz</i>	1
Role of Endothelin Receptor Antagonists in the Treatment of Pulmonary Arterial Hypertension <i>Steven H. Abman</i>	13
Oral Iron Chelators <i>Maria Domenica Cappellini and Paolo Pattoneri</i>	25
The Treatment of Hyperhomocysteinemia <i>Bradley A. Maron and Joseph Loscalzo</i>	39
Stroke Rehabilitation: Strategies to Enhance Motor Recovery <i>Michael W. O'Dell, Chi-Chang David Lin, and Victoria Harrison</i>	55
Cardiomyopathic and Channelopathic Causes of Sudden Unexplained Death in Infants and Children <i>David J. Tester and Michael J. Ackerman</i>	69
Bisphosphonate-Related Osteonecrosis of the Jaw: Diagnosis, Prevention, and Management <i>Salvatore L. Ruggiero and Bhoomi Mehrotra</i>	85
IL-23 and Autoimmunity: New Insights into the Pathogenesis of Inflammatory Bowel Disease <i>Clara Abraham and Judy H. Cho</i>	97
Necrotizing Enterocolitis <i>Marion C.W. Henry and R. Lawrence Moss</i>	111
Cancer Screening: The Clash of Science and Intuition <i>Barnett S. Kramer and Jennifer Miller Croswell</i>	125
Biomarkers for Prostate Cancer <i>Danil V. Makarov, Stacy Loeb, Robert H. Getzenberg, and Alan W. Partin</i>	139
Management of Breast Cancer in the Genome Era <i>Phuong Khanh H. Morrow and Gabriel N. Hortobagyi</i>	153

MicroRNAs in Cancer <i>Ramiro Garzon, George A. Calin, and Carlo M. Croce</i>	167
Erythropoietin in Cancer Patients <i>John A. Glaspy</i>	181
Thrombopoietin and Thrombopoietin Mimetics in the Treatment of Thrombocytopenia <i>David J. Kuter</i>	193
Evolving Treatment of Advanced Colon Cancer <i>Neil H. Segal and Leonard B. Saltz</i>	207
Barrett's Esophagus and Esophageal Adenocarcinoma <i>Robert S. Bresalier</i>	221
Primary Myelofibrosis: Update on Definition, Pathogenesis, and Treatment <i>Omar I. Abdel-Wahab and Ross L. Levine</i>	233
Nicotine Dependence: Biology, Behavior, and Treatment <i>Riju Ray, Robert A. Schnoll, and Caryn Lerman</i>	247
Food Allergy: Recent Advances in Pathophysiology and Treatment <i>Scott H. Sicherer and Hugh A. Sampson</i>	261
Immunomodulation of Allergic Disease <i>David H. Broide</i>	279
Hypereosinophilic Syndrome: Current Approach to Diagnosis and Treatment <i>Amy Klion</i>	293
Extensively Drug-Resistant Tuberculosis: A New Face to an Old Pathogen <i>Sheela Shenoi and Gerald Friedland</i>	307
Polycystic Kidney Disease <i>Peter C. Harris and Vicente E. Torres</i>	321
The Kidney and Ear: Emerging Parallel Functions <i>Elena Torban and Paul Goodyer</i>	339
The Expanded Biology of Serotonin <i>Miles Berger, John A. Gray, and Bryan L. Roth</i>	355
Advances in Autism <i>Daniel H. Geschwind</i>	367
Chronic Consciousness Disorders <i>James L. Bernat</i>	381

Goals of Inpatient Treatment for Psychiatric Disorders <i>Steven S. Sharfstein</i>	393
Understanding and Reducing Variation in Surgical Mortality <i>John D. Birkmeyer and Justin B. Dimick</i>	405
MRI-Guided Focused Ultrasound Surgery <i>Ferenc A. Jolesz</i>	417
Genetic Testing in Clinical Practice <i>Steven W.J. Lamberts and André G. Uitterlinden</i>	431
The HapMap and Genome-Wide Association Studies in Diagnosis and Therapy <i>Teri A. Manolio and Francis S. Collins</i>	443
Prospects for Life Span Extension <i>Felipe Sierra, Evan Hadley, Richard Suzman, and Richard Hodes</i>	457
Emerging Concepts in the Immunopathogenesis of AIDS <i>Daniel C. Douek, Mario Roederer, and Richard A. Koup</i>	471
Lessons Learned from the Natural Hosts of HIV-Related Viruses <i>Mirko Paiardini, Ivona Pandrea, Cristian Apetrei, and Guido Silvestri</i>	485

Indexes

Cumulative Index of Contributing Authors, Volumes 56–60	497
Cumulative Index of Chapter Titles, Volumes 56–60	501

Errata

An online log of corrections to *Annual Review of Medicine* articles may be found at
<http://med.annualreviews.org/errata.shtml>