MicroRNAs in Cancer

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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.

Key Words

tumors, oncogenes, tumor suppressors, outcome, treatment
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, immune- 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 miRNAs—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
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
### Table 1  MiRNAs with experimental data supporting a tumor suppressor or oncogene function in cancer

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

*Abbreviations: 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 miRNAs 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 antipapoptotic 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 of two miRNAs, miR-17-92, estab-
lishing 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 suppressor gene TP53 (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 \textit{c-myc} transactivates miRNAs, in particular the \textit{miR-17-92} cluster (63). Recent work also suggests that \textit{c-myc} negatively regulates transcription of tumor suppressor miRNAs, such as \textit{let-7} (\textit{let-7a-1}, \textit{let-7f-1}, \textit{let-7d}, \textit{let-7c} and \textit{let-7g}) and \textit{miR-29} family members (\textit{a}, \textit{b}, and \textit{c}) (72). Chromatin immunoprecipitation experiments showed that \textit{c-myc} binds to conserved sequences of the miRNA promoter that it represses. Functionally \textit{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 \textit{c-myc}, \textit{E2F}, and \textit{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 \textit{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 \textit{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 \textit{miR-29} family, which includes targets \textit{DNMT3A} and \textit{3B}, induces global DNA hypomethylation and tumor suppressor gene re-expression in lung cancer (38). Interestingly, \textit{miR-29a} is downregulated in lung cancer patients, and an inverse correlation was found between \textit{miR-29b} and \textit{DNMT3B} expression, suggesting that the downregulation of this miRNA may contribute to increased \textit{DNMT3} levels, as well as hypermethylation 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 \textit{miR-155}, \textit{miR-221}, and \textit{miR-222} and low levels of \textit{miR-29c}) was clearly associated with time to progression in CLL (15). In lung cancer, low levels of \textit{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 \textit{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 \textit{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

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Disease</th>
<th>Expression in poor outcome</th>
<th>Variable</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7a</td>
<td>non–small cell lung cancer</td>
<td>low</td>
<td>OS^a</td>
<td>16, 75</td>
</tr>
<tr>
<td>miR-21</td>
<td>colon adenocarcinoma</td>
<td>high</td>
<td>OS, DFS^a</td>
<td>77</td>
</tr>
<tr>
<td>miR-21</td>
<td>CLL^2</td>
<td>high</td>
<td>time to progression</td>
<td>15</td>
</tr>
<tr>
<td>miR-155</td>
<td>CLL</td>
<td>high</td>
<td>time to progression</td>
<td>15</td>
</tr>
<tr>
<td>miR-221/222</td>
<td>CLL</td>
<td>high</td>
<td>time to progression</td>
<td>15</td>
</tr>
<tr>
<td>miR-146</td>
<td>CLL</td>
<td>high</td>
<td>time to progression</td>
<td>15</td>
</tr>
<tr>
<td>miR-29-c</td>
<td>CLL</td>
<td>low</td>
<td>time to progression</td>
<td>15</td>
</tr>
<tr>
<td>miR-196-a</td>
<td>pancreas adenocarcinoma</td>
<td>high</td>
<td>OS</td>
<td>76</td>
</tr>
<tr>
<td>miR-191</td>
<td>AML^2</td>
<td>high</td>
<td>OS, DFS</td>
<td>38</td>
</tr>
<tr>
<td>miR-199a</td>
<td>AML</td>
<td>high</td>
<td>OS, DFS</td>
<td>38</td>
</tr>
<tr>
<td>miR-181a/b</td>
<td>CN-AML (&lt;60 years)</td>
<td>low</td>
<td>DFS</td>
<td>78</td>
</tr>
<tr>
<td>miR-181a</td>
<td>CLL</td>
<td>high</td>
<td>time to progression</td>
<td>15</td>
</tr>
</tbody>
</table>

^a Abbreviations: 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 antagonirs) 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 nonhuman 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.

**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.

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