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Keynote Lecture

MiRNAs and Cancer

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Cancer is the result of a complex multistep process that involves the accumulation of sequential alterations of several genes, including those encoding microRNAs (miRNAs). miRNAs are a class of 17- to 27-nucleotide single-stranded RNA molecules that regulate gene expression posttranscriptionally. A large body of evidence implicates aberrant miRNA expression patterns in most, if not all, human malignancies. This article reviews our current knowledge about miRNAs, focusing on their involvement in cancer and their potential as diagnostic, prognostic, and therapeutic tools. ***(Am J Pathol 2009, 174:1131–1138; DOI: 10.2353/ajpath.2009.080794)***

Cancer, which develops because of a multistep process resulting in the accumulation of several genomic alterations, is characterized by unrestricted proliferation, invasion, and metastasis. In cancer, many molecular pathways are affected, involving canonical protein-coding genes as well as recently discovered noncoding genes. Noncoding RNAs include a class of small RNAs (17 to 27 nucleotides in length), microRNAs (miRNAs), that control gene expression by regulating mRNA translation.

The biogenesis of miRNAs starts with the transcription of genomic regions located within or between protein-coding genes, resulting in the synthesis of miRNA precursor molecules (pri-miRNAs). Pri-miRNAs are currently thought to be transcribed primarily by RNA polymerase II and, less frequently by RNA polymerase III. Drosha, a specific ribonuclease of the RNase III endonuclease family, then enzymatically cuts the transcribed pri-miRNA in a smaller fragment (~70 nucleotides). This hairpin pre-miRNA is then exported to the cytoplasm by Exportin-5 in a Ran-GTP-dependent manner and cleaved into an imperfect double-strand RNA (dsRNA), duplex-designated miRNA, which is termed miRNA/miRNA*. This process is performed by Dicer, an RNase III endonuclease composed of a helicase domain and a dsRNA-binding domain. One strand of the miRNA/miRNA* duplex is then selected to function as a mature miRNA and preferentially loaded into a miRNA ribonucleoprotein (miRNP) complex, whereas the other strand is likely degraded.[1] As a part of the miRNP complex, single or multiple miRNA copies bind to mRNA 3′untranslated regions (3′UTR). Those that bind with perfect complimentarity lead to mRNA degradation; whereas imperfect binding leads to inhibition of translation (Figure 1).

The numerous biochemical mechanisms that govern miRNA function, however, are likely dependent on the availability of local regulatory factors.[2] An estimated one-third of protein-coding human mRNAs are susceptible to this complex miRNA regulatory network. Every cellular process is regulated by miRNAs, and an aberrant miRNA expression signature is a hallmark of several diseases, including cancer. These data suggest that miRNA genes could function as potential oncogenes and tumor repressors genes in the human body. Thus, an accurate evaluation of changes in miRNA expression could provide new insight into basic mechanisms of cancer.

Methods to Identify Deregulated Expression of miRNAs in Human Tumors

The first evidence of aberrant miRNA expression in human cancers was described in B-cell chronic lymphocytic leukemia, wherein hemizygous and/or homozygous chromosomal deletion at the 13q14 locus resulted in the loss or reduction of *miR-15* and *miR-16* expression.[3] This discovery of new genes linked to cancer prompted investigation of miRNA expression in human tumors. Consequently, several techniques have been developed to support this research.

Two widely performed high-throughput techniques are used for miRNA profiling. The solid-phase array-based platform, developed first by Liu and colleagues,[4] is semi-quantitative, requires transcript amplification/labeling,

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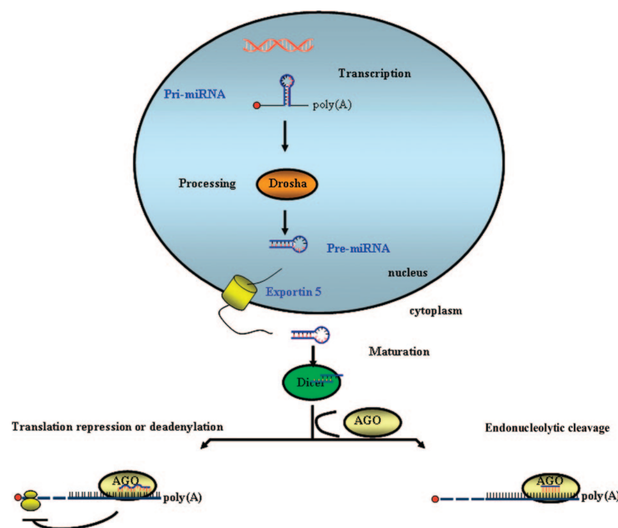

Figure 1. Biogenesis of miRNAs and assembly into protein complexes. miRNA precursor molecules (pri-miRNAs) fold into hairpin structures that contain imperfect base-paired stems in a two-step process catalyzed by two different RNase III-type endonucleases. Drosha first cleaves pri-miRNAs, forming ~70 nucleotide hairpins (pre-miRNAs) in the nucleus. Subsequently, pre-miRNAs are transported to the cytoplasm by Exportin 5, where they are cleaved by Dicer to yield ~20-bp miRNA duplexes. One miRNA strand is then selected to function as a mature miRNA, whereas the other strand is degraded. After processing, miRNAs are assembled into RNP (ribonucleic protein) complexes, called miRNPs, with proteins of the AGO family. miRNPs tether to the 3′UTR of a mRNA target to repress protein synthesis. In the case of perfect bp alignment, the miRNP complex cleaves the duplex miRNA-mRNA; however, multiple mechanisms[2] are used on duplex miRNA:mRNA with imperfect complementary.

and carries an inherent limitation of cross-hybridization among miRNAs of the same family. Conversely, flow-based, liquid-phase profiling has the advantage of increased specificity in discriminating the expression of closely related miRNAs as well as higher sensitivity in detecting modest decreases in down-regulated miRNAs. However, flow-based, liquid-phase profiling is technically demanding with respect to quality consistency in the production of miRNA probes.[5] Data obtained by either of these two methods needs to be validated independently by a second technique, such as Northern blot or quantitative real-time polymerase chain reaction, to confirm the miRNA expression profile. The use of these techniques revealed aberrant miRNA expression in numerous tumors when they were compared with their normal counterparts, thereby suggesting that a link does exist between miRNAs and cancer (Table 1).[6–14]

Cause of Abnormal miRNA Expression

MiRNA expression can be altered by several mechanisms in human cancer including chromosomal abnormalities, epigenetic changes, mutations and polymorphisms (SNPs), and defects in the miRNA biogenesis machinery.

Chromosomal Abnormality

MiRNAs often reside in particular genomic regions that are prone to alterations in cancer. These regions could include either a minimal region of loss of heterozygosity, which can harbor a tumor suppressor gene; a minimal region of amplification, which might contain oncogenes; or fragile sites. Fragile sites are preferential sites of sister chromatid exchange, translocation, deletion, amplification or integration of plasmid DNA, and insertion of tumor-associated viruses such as human papilloma virus.[15]

The high frequency of genomic alterations in miRNA loci was recently confirmed by an extensive study of high-resolution array-based genomic hybridization on 227 human ovarian cancer, breast cancer, and melanomas samples.[16] The findings of this study proved that miRNA expression correlated with miRNA copy number, and these data tightly overlap with the miRNA expression data analyzed on a set of breast cancer samples in an independent study.[9]

Specific examples of miRNAs located in instable genomic regions include the *miR-15a/16* cluster, embedded into 13q14, and both *miR-143* and *miR-145,* located at 5q33. In fact, B-cell chronic lymphocytic leukemias and pituitary adenomas, which often harbor 13q14 deletions, showed a decreased expression of *miR-15a* and *miR-16*,[3,7,10] whereas deletion of the 5q33 region observed in lung cancer seems to contribute to the decreased levels of *miR-143* and *miR-145* in this tumor.[15] Conversely, cluster *miR-17-92,* located at chromosome 13q31, a region amplified in B-cell lymphomas[17] and lung cancers,[18] has been found to be overexpressed in these malignancies. Overall, these findings suggest that the location of miRNAs in a genomic region amenable to alterations is not a random event, thereby indicating that the loss or the gain of genomic regions including miRNAs in a specific type of cancer could participate to the cause of this malignancy.

Epigenetic Changes

Recent findings indicate that epigenetic aberrations affect miRNA expression. An extensive analysis of genomic sequences of miRNA genes showed that approximately half of these genes are associated with CpG islands, suggesting that miRNAs can represent candidate targets of the DNA methylation machinery. Analysis of several miRNA-associated CpG islands in five cell lines also indicated that miRNA gene methylation is detectable at high frequencies, both in normal and malignant cells.

Methylation status could possibly explain the deregulated expression of miRNAs in cancer.[19] Some examples have been reported by Iorio and colleagues.[8] They found that the treatment of a human ovarian cancer cell line (OVCAR3) with the demethylating agent 5-aza-2′deoxycytide (5-AZA-CdR) increased the expression levels of nine miRNAs. Interestingly, three of nine miRNAs, *miR-21, miR-203*, and *miR-205,* have been found to be also overexpressed in ovarian carcinomas when compared with their normal counterparts, suggesting that hypomethylation could be the mechanism responsible for their overexpression *in vivo*.[8] Moreover, *miR-21* and *miR-203* are embedded in a region associated with CpG islands, whereby the DNA methylation machinery could directly

Table 1. Selected miRNAs Aberrantly Expressed in Tumors

		Selected miRNAs		
Organ	Disease type	Up-regulated	Down-regulated	References
Liver	HCA and FNH*	224	122a, 422b, 203, 200c	6
	HCC*†	21, 224, 10b, 221, 222, 20, 18	199a, 199b, 200b, 223, 122, 214,145, 150	6
	Cholangiocarcinomas*	21, 23a, 141, 200b, 27a		6
Pancreas	PET*	23a, 342, 26a, 30d, 26b, 103, 107	155, 326, 339, 326	6
	Insulinomas*	203, 204, 211,		6
	PACC*	23a, 342, 26a, 30d, 26b, 103, 105	155, 326, 339,326	6
	Ductal adenocarcinomas*†	21, 221, 181a, 155, 222, 181b, 107	148a, 375	6
Esophagus	ESCC*	25, 424, 151	100, 99, 29c, 140, 205,203, 202	6
Stomach	Adenocarcinomas*†	21, 223, 25, 17-5p, 125b, 181b, 106a, 107, 92, 103, 221, 93, 100, 106b	136, 218, 212, 96, 339	6
Colon	Adenomas*	21		6
	Adenocarcinomas*†	21, 92, 20a, 106a, 92, 223, 203		6
	Adenocarcinomas stage II*		145	6
Hematopoietic tissue	CLL*	190, 33, 19a, 140, 123, 10b, 92, 188, 154, 217, 101, 196, 134, 141, 132, 192, 16, 15	181b, 220	7
Ovary	Carcinomas* (serous, clear cell, endometrioid)	200a, 200c	let-7d, 100, 101, 105, 125a, 125b, 126, 133a, 137, 140, 143, 147, 199a, 199b, 224, 9, 9*, 99a	8
Breast	Carcinomas*	155, 21	125b, 145, 10b	9
Lung	NSCLC†	21, 191, 155, 210	126*, 224	
Pituitary gland	Adenoma*	15, 16		10
Prostate	Carcinomas†	32, 182, 31, 26a, 200c	520h, 494, 490, 133a, 1, 218, 220, 128a	11
Thyroid	Papillary carcinomas*	221, 221, 146a, 181b		12, 13
	Anaplastic carcinomas*		30 days, 125b, 26a, 30a-5p	14

HCA, hepatocellular carcinomas; FNH, focal nodular hyperplasia; PET, pancreatic endocrine tumors; PACC, pancreatic acinar cell carcinomas; ESCC, esophageal squamous cell carcinomas; CLL, chronic lymphocytic leukemia; NSCLC, non-small cell lung cancer.
*miRNA comparative analysis: nontumor or tumor tissue versus normal tissue.
†miRNA comparative analysis: tumor tissue versus adjacent nontumor tissue.

affect the expression of these miRNAs.[8] Conversely, decreased *miR-124a* expression was attributed to DNA hypermethylation in colon, breast, and lung carcinomas.[20]

Mutations and SNPs

Mutations and polymorphisms located in mature miRNA, pre-miRNA, or more likely in adjacent genomics regions can also change miRNA expression by affecting their processing. These events in miRNAs are rarer than in mRNA protein coding genes, because the size of miRNAs and their precursors is small. A few cases have been already reported in the literature, however.

Inherited mutations in the primary transcripts of *miR-15a* and *miR-16–1* have been reported to be responsible for low expression levels *in vitro* and *in vivo*. Decreased expression of these miRNAs in familial chronic lymphocytic leukemia and familial breast cancer was also associated with deletion of the normal allele encompassing *miR-15a* and *miR-16–1*.[21] The importance of this mutation was further supported in a spontaneous mouse model of chronic lymphocytic leukemia.[22]

MiRNA genomic region can also include SNPs. Hu and colleagues,[23] conducted a systematic survey of common pre-miRNA sequences and their surrounding regions and evaluated in detail the association of four selected SNPs in four miRNAs (*miR-146a*, *miR-196a2*, *miR-499*, and *miR-149*) with the survival of individuals with non-small cell lung cancer. They found that patients with non-small cell lung cancer carrying a variant homozygote of the SNP located in the 3p miRNA region of *miR-196a-2* had poor survival, possibly through a mechanism of elevated expression of mature *miR-196**.[23] These findings suggest that SNPs located in miRNA regions may be prognostic biomarkers of certain malignancies.

Defects in the miRNA Biogenesis Machinery

Despite normal expression levels of pri-miRNA, a number of human primary cancers displayed reduced levels of

mature miRNAs. Thomson and colleagues[24] explained this difference as a processing defect because of the loss of the RNase III Drosha. Conversely, targeted Drosha activity, directed by the *ALL1(MLL*) fused gene, appeared to be responsible for *miR-191* up-regulation in human acute lymphoblastic leukemia.[25] In addition, decreased Dicer endonuclease activity in a proportion of non-small cell lung cancers, correlated with reduced *let-7* expression, unfavorable postoperative survival, and poor tumor differentiation status.[26] The loss of Dicer likely represents a somatic alteration because Dicer-deficient mice failed to thrive beyond gastrulation because of the lack of multipotent stem cell development.[27] Finally, changes in miRNA expression can be attributable also to either the frequent deregulation of transcription factors in cancer or viruses that often integrate within the DNA of tumoral cells.[28,29]

Significance of the Altered miRNA Expression in Tumors

Oncogenes

High-throughput analyses have reported altered miRNA expression in all tumors investigated to date, suggesting that miRNAs might be implicated in tumorigenesis, likely by regulating oncogene or tumor suppressor genes. The *miR-17-92* polycistron represents the first example of miRNA acting as a mammalian oncogene. This cluster is embedded in a human genomic locus, 13q31.3, which is a region that is amplified in several types of lymphoma and solid tumors. Expression of the *miR-17-92* cluster in the *Eμ-myc* transgenic mouse model of B-cell lymphoma accelerated disease onset and progression. In these mice, transcription of the *miR-17-92* cluster was directly transactivated by c-Myc, a transcription factor that is frequently overexpressed in cancer cells, through its direct interaction with the putative promoter region.[30,31] The *miR-17-92* cluster and its paralog, *miR-106b-25*, seem to be tightly linked to the functions of the E2F family of transcription factors as well. Indeed, members of both clusters target E2F1 and in turn, can be activated by E2F, establishing a negative feedback loop.[28]

Although these data support a role for the *miR-17-92* cluster in promoting tumorigenesis, there is also evidence suggesting that loss-of-function of these miRNAs might be advantageous for cancer cells. Loss-of-heterozygosity at the 13q31.3 locus has been reported in ovarian cancers, breast cancers, and melanomas. Consistent with these observations, introduction of *miR-17* into breast cancer cell lines reduced their proliferation.[32] This effect was attributable in part to the inhibition of the *amplified in breast cancer 1* (*AIB1*) gene, which encodes a transcriptional co-activator of the estrogen receptor and E2F1. Thus, the same miRNA can have both pro- and anti-tumorigenic activities depending on the targets available in a given cellular context.

The first direct example that a single miRNA has an oncogenic role is *miR-155*, the overexpression of which has been linked to several types of lymphomas such as Hodgkin and Burkitt lymphoma.[33] Recently, Costinean and colleagues[34] demonstrated the role of this miRNA in tumorigenesis by producing transgenic mice that specifically overexpress *miR-155* in B cells. These transgenic mice developed a preleukemic lymphoproliferative disease that progressed to B-cell leukemia and high-grade lymphoma. A microarray study of the malignant B cells isolated from *miR-155* transgenic mice compared with nontransgenic control reported several deregulated protein-coding genes in these cells, potentially identifying direct and indirect targets of *miR-155.* These findings suggest that *miR-155* deregulation could be an early event in oncogenesis, which needs additional genetic alterations for the development of the fully malignant phenotype.

Cell Cycle Regulation

MiRNAs can also contribute to tumorigenesis by modulating the expression of proteins that directly regulate circuitry controlling cellular life and death decisions. *MiR-221/222*, up-modulated in many tumors, has been reported to target $p27^{Kip1}$ and $p57^{Kip2}$ proteins,[35,36] two negative cell-cycle regulators that bind to Cdk/cyclin complexes and inhibit the G_1/S phase switch. Similarly, members of the *miR-17-92* and *miR-106b-25* clusters promote cell proliferation by targeting $p21^{Waf/Cip1}$, which is involved in the same checkpoint.[28]

Conversely, down-modulated miRNAs in cancer control cell cycle by reducing cyclin and/or Cdk levels. For instance, *miR-122*, which has been reported to be down-regulated in hepatocellular carcinomas, targets cyclin G1, whose levels are increased in hepatocellular carcinomas and experimental models of hepatocarcinogenesis. Loss of cyclin G1 is associated with a significantly lower tumor incidence after carcinogen treatment.[37] Similarly, the *mir-15a/16* cluster reduces the levels of cyclin D1, cyclin D3, cyclin E1, and CDK6, suggesting that reduced levels of the miR-16 family trigger an accumulation of multiple cell cycle-promoting genes.[38] In addition, these two miRNAs also contribute to apoptosis; they decrease the levels of the apoptotic inhibitor Bcl-2. One report demonstrated that high levels of this protein correlated with low levels of the *miR-15a/16* cluster in chronic lymphocytic leukemia.[39] Members of the *miR-17-92* and *miR-106b-25* clusters have also been reported to participate in apoptosis by inhibiting the proapoptotic factor Bim.[28,40]

Progression and Metastasis

Moreover, a role for miRNAs has been established in the later steps of tumorigenesis, progression, and metastasis. Several miRNAs, such as *miR-21* and *miR-10b,* seem to play an important role. *MiR-21* functions as an oncogene and contributes to tumorigenesis, in part through regulation of the tumor suppressor gene tropomyosin 1 (TPM1), a protein involved in cell migration. Recent studies reported that the suppression of *miR-21* in metastatic breast cancer cells or malignant hepatocytes signifi-

cantly reduced invasion and metastasis. Two newly discovered *miR-21* targets, programmed cell death 4 (PDCD4) and maspin, have been implicated in these events.[41]

In another study, Ma and colleagues[42] found that the expression of *miR-10b* was increased in metastatic breast cancer cells compared with healthy or nonmetastatic tumorigenic cells. They also investigated the cause of this up-regulation and identified the transcription factor (Twist) as the inducer of *miR-10b* overexpression. The authors further validated HOXD10 as a target of *miR-10b* and showed that a decrease in HOXD10 levels resulted in higher levels of RHOC, which stimulates cancer cell motility.[42]

MiRNA Applications

miRNAs as Diagnostic and Prognostic Tools

The accumulated data on miRNA expression levels in tumors demonstrate that miRNAs are promising candidates to distinguish different tumors and different subtypes of tumors as well as to predict their clinical behavior. Two large studies have supported the role of miRNAs as either prognostic and/or diagnostic markers. Lu and colleagues analyzed 334 samples, including samples from multiple human cancers. They observed that miRNA expression profile clusters samples by the developmental origin of the tissue. For instance, tissues of epithelial origin cluster separately than tissues of the other origin.[5] Moreover, examining the miRNA profile of bone marrow samples from patients with acute lymphoblastic leukemia, they noted that miRNA profile can accurately distinguish tumors reflecting a different mechanism of transformation. Indeed, acute lymphoblastic leukemia samples with different rearrangements, such as mixed lineage leukemia, *BCR/ABL*, or *TEL/AML1*, clustered nonrandomly. Tumors of the gastrointestinal tract were also interesting because they all clustered together, reflecting their common derivation from tissue of embryonic endoderm.

Another interesting observation was that miRNAs correlate with cellular differentiation stages. In the same report, they explored also an experimental model in which they treated myeloid leukemia cell line HL-60 with all-trans retinoic acid, a potent inducer of neutrophilic differentiation. MiRNA profiling revealed that the induction of many miRNAs was coincident with the different stages of differentiation. In a different study, Volinia and colleagues,[43] by hierarchical clustering analysis of 540 samples including 363 from six of the most frequent solid tumors types (breast, colon, lung, pancreas, stomach, and prostate) and 177 normal controls, showed that a unique miRNA signature enabled the tumor samples to be grouped on the basis of their tissue of origin. Several other miRNA profiling studies have been performed on various cancer types highlighting also the prognostic role of these small RNAs in large set of samples. For example, Murakami and colleagues[44] reported that *miR-222, miR-106a*, and *miR-17-92* clusters have been associated with the degree of the differentiation of hepatocellular carcinomas; whereas high expression of *miR-21* was associated with poor survival of patients with incidence of pancreas endocrine tumors[45] or colon adenocarcinomas.[46] This suggests a role for specific miRNAs for identifying disease progression.

On these bases, miRNA profiling has acquired importance in resolving one of the most demanding issues in cancer diagnostics—the origin of metastasis of unknown primary tumor. In a recent study, a miRNA-based tissue classifier was constructed to identify the tissue of origin of metastatic tumors.[47] Three-hundred thirty-three formalin-fixed, paraffin-embedded samples, including 205 primary tumors and 131 metastatic tumors, were analyzed on a custom microarray platform and used for this analysis. Briefly, the researchers built the classification algorithm as a branched binary tree: in each node of the tree, classification proceeds to one of two possible branches, grouping together tissues with underlying similarities. The decision at each node is based on level expression of only 48 miRNAs with potential specificity in tissue differentiation and embryogenesis. This allows each cancer type to be assigned to one of two possible branches of the tree. Primary tumors and metastasis from the same tissue were grouped together because no significant differences were observed in miRNA expression. This classifier emerges as more accurate than those using mRNA expression profiling and represents a remarkable advance for determining the origin of metastatic cancer of unknown primary origin.

miRNAs as Therapeutic Tools

Because of the significance of miRNAs in cancer, the management of miRNAs with altered expression in cancer should be considered as a therapeutic strategy. Several reports have demonstrated that miRNAs can be responsible for drug resistance as well.[28,48] Thus, miRNAs or anti-microRNA (anti-miRNA) may be used in therapeutics either individually or in combination with other treatments that have lost efficacy. The delivery modality of these molecules critically affects the success of the experimental approach. Hence, several tools have been developed to enable selective targeting of miRNA pathways, which include tools that can either reconstitute reduced miRNAs or reduce overexpressed miRNAs. Recently, technologies have also been described that permit selective protection from miRNAs by limiting accessibility to miRNA binding sites.

Re-Expression of miRNAs

Restoring miRNA expression in diseases in which expression is consistently reduced could be a novel therapeutic approach. Several techniques have been developed to deliver miRNAs in *in vitro* systems. One of these techniques involves synthetic small RNAs called miRNA mimetics, which contain the exact sequence of the endogenous miRNA. MiRNA mimetics are delivered as perfect complementary duplexes to improve RISC loading of

miRNA. Moreover, miRNA mimetics can be modified to have enhanced efficiency by increasing the affinity for a specific target and by reducing other unwanted miRNA effects.[49] Another approach to increase expression of specific miRNAs involves introduction of DNA vectors that contain artificial miRNA precursor sequences for miRNA expression. In this case, the mechanism of the silencing of the mRNA target seems to be through mRNA degradation and not translational repression.[50] Alternatively, viral vectors might also be used to deliver miRNAs in *in vitro* systems. Although the devices described above have strong potential to be used in therapy, further studies are necessary to demonstrate that these methods can really operate efficiently by delivering miRNAs in *in vivo* models.

Anti-miRNAs

Antisense oligonucleotides that bind directly to miRNAs and block their activity are generally named anti-miRNAs. They work by stoichiometric interaction with mature miRNAs, either titrating them from biologically active pools of mature miRNAs or binding to miRNA precursors and inhibiting the biogenesis of mature miRNAs. Early reports describe 2′*O*-methyl RNA oligonucleotides as efficient anti-miRNAs because the 2′*O*-methyl group increases the stability of the anti-miRNA molecules. Subsequently, several chemical modifications have been carried to these oligonucleotides to improve their efficacy.[51] *In vitro* experimental procedures demonstrated a powerful inhibitory activity of 2′-*O*-methoxyethyl oligonucleotides (2′-MOEs), in which every third nucleotide was substituted with a locked nucleic acid (LNA) residue; and of the 2′-fluoro-oligonucleotide with a phosphorothioate backbone.[52]

The first example of an *in vivo* knockdown of miRNA has been achieved by using the antagomiR to probe the liver-specific *miR-122*.[53] The antagomiR is a 2′*O*-methylated oligonucleotide with a cholesterol molecule linked at its 5′ end that improves the cellular uptake of this molecule. However, in two similar studies, Esau and colleagues[54] and Elmen and colleagues[55] reported that unconjugated 2′-MOE anti-miR-122 and LNA-antimiR-122 also effectively antagonize the liver-expressed *miR-122* in nonhuman primates.

Ebert and colleagues[56] describe another approach to inhibit the function of specific miRNAs in cells. They used synthetic mRNAs that contained multiple binding sites for an endogenous miRNA. These synthetic mRNAs, or miRNA sponges, bind up the miRNA and prevent its association with its endogenous targets. In culture cells they were at least as effective as LNA anti-miRNAs. Anti-miRNA therapy holds great promise, but care must be taken to confirm that it does not deregulate genes that are not an intended point of therapy.

Target Protectors

A miRNA:mRNA pair usually possesses perfect complementarity between nucleotides 2 and 8 from the 5′ end of the miRNA (seed region). Target protectors (TPs) are small oligonucleotides with perfect complementary to the seed region and to 5′ and 3′ flanking sequences in the 3′ UTR of specific miRNA target genes. Thus, TPs prevent miRNA access to those sites. These specialized oligonucleotides have recently been reported to interfere with *miR-430*-mediated repression of specific 3′ UTRs in zebrafish.[57] Therefore, TPs are advantageous in cases in which the mRNA targets of miRNAs are known. In the years to come, as more and more targets of miRNAs are validated, this technology will become very useful to affect only those miRNA target genes intended as point of therapy.

Conclusion

miRNAs participate in keeping the balance of genes regulating networks that determine the cells' fate. Deregulation of miRNAs, which is a frequent outcome in human cancer, seriously weakens this balance, thereby contributing to oncogenesis and cancer progression. Several studies have reported that miRNAs affect the expression of genes and pathways involved in cancer pathogenesis from initiation to metastasis disease. However, more research is needed to validate these findings, and murine models genetically modified by deleting or overexpressing putative tumor-suppressor or oncogenic miRNAs could help to identify miRNAs regulating cancer-related pathways. Therapeutics can take advantage of this new knowledge as can diagnostics and prognostics: clinical applications exploiting our understandings of miRNA function will be the next great challenge in cancer research.

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