Molecular Pathways: MicroRNAs as Cancer Therapeutics
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Abstract
MicroRNAs (miRNA) are approximately 18 to 25 nucleotides in length and affect gene expression by silencing the translation of messenger RNAs. Because each miRNA regulates the expression of hundreds of different genes, miRNAs can function as master coordinators, efficiently regulating and coordinating multiple cellular pathways and processes. By coordinating the expression of multiple genes, miRNAs are responsible for fine-tuning the cell’s most important processes, like the ones involved in cellular growth and proliferation. Dysregulation of miRNAs appears to play a fundamental role in the onset, progression and dissemination of many cancers, and replacement of downregulated miRNAs in tumor cells results in a positive therapeutic response. Thus, in theory, inhibition of a particular miRNA linked to cancer onset or progression can remove the inhibition of the translation of a therapeutic protein—and conversely, administration of a miRNA mimic can boost the endogenous miRNA population repressing the translation of an oncogenic protein. Although several basic questions about their biologic principles still remain to be answered, and despite the fact that all data with respect to miRNAs and therapy are still at the preclinical level, many specific characteristics of miRNAs in combination with compelling therapeutic efficacy data have triggered the research community to start exploring the possibilities of using miRNAs as potential therapeutic candidates. Clin Cancer Res; 18(16); 4234–9. ©2012 AACR.

Background
Recent research has shown us that the nonprotein-coding portion of the genome is crucial for gene expression regulation in a normal as well as disease setting. The functional relevance of this fragment of the genome is particularly evident for a class of small noncoding RNAs called microRNAs (miRNA). miRNAs are a class of small, evolutionarily conserved, noncoding RNAs of 18 to 25 nucleotides in length that posttranscriptionally control the translation of mRNAs (1). The number of miRNAs is growing rapidly, and more than 700 miRNA genes have already been identified in the human genome alone, which approaches approximately 3% of the number of all human genes (Sanger miRBase). miRNAs are predicted to regulate the translation of more than 60% of protein-coding genes, and thus they coordinate many processes, including proliferation, development, differentiation, and apoptosis (2). Thus, miRNAs constitute one of the most abundant classes of gene-regulatory molecules in animals. Because of their involvement in all cellular processes, the abnormal expression or alteration of miRNAs contributes to a range of human malignancies, including cancer. The biogenesis of miRNAs is a multistep process that is closely related to their regulatory functions. The biosynthesis starts in the nucleus of the cell following transcription, where a precursor miRNA (pre-miRNA) is produced through the action of Drosha and further continues through the cytoplasm, where Dicer processes it to the mature functional miRNA with the ability to silence target mRNA translation (Fig. 1; refs. 3, 4).

Interestingly, a wide range of miRNAs that map to regions of the human genome are known to be frequently deleted or amplified in cancer (5). This finding suggests that miRNAs could contribute to the development of cancer and has opened a new area of research for miRNA dysregulation in human cancer. Subsequently miRNAs were shown to be differentially expressed in cancer cells, in which they formed unique miRNA expression patterns (6). Dysregulation of miRNAs in cancer can occur through epigenetic changes and genetic alterations, which can affect the production of the primary RNAs, their processing to their mature miRNA form, and/or interactions with miRNA targets. The recent findings of genetic defects in cancer-associated genes of the miRNA processing machinery, such as TARBP2 (7), Dicer (8), and XIPO5 (9), has strongly highlighted the relevance of these pathways in cellular transformation, where such defects contribute to a global dysregulation of miRNAs in cancer. Although miRNAs have been categorized as oncogenic or tumor suppressive, the miRNA expression profile of human tumors is characterized by the impairment of miRNA production, which results in global miRNA...
downregulation (6–10). The first high-throughput study accessing miRNA expression profiles in cancer was done in 334 patient samples of various cancer types and showed that miRNA expression profiles can distinguish the developmental lineage and differentiation state of the tumors (6). In fact, miRNA profiling can be more accurate at classifying tumors than mRNA profiling because miRNA expression correlates closely with tumor origin and stage (6). Further studies were able to identify the tissue of origin of metastatic tumors with unknown primary origin based on the miRNAs' expression profiles (6). Nonetheless, the detection of global miRNA expression patterns for the diagnosis of cancer has not yet been proved despite of the promising results of some individual or small groups of miRNAs, like the combination of high miR-155 and low let-7 expression in non–small cell lung cancer that correlates with poor prognosis (11).

Much of what we have learned concerning the functional contribution of specific miRNAs to cancer development is based on studies using germline transgenic and knockout mice. Several strains of mice silencing or overexpressing cancer-associated miRNAs have been developed and characterized in an in vivo context recapitulating the behavior of some human malignancies, which can be extremely useful to evaluate therapeutics. Perhaps the most exciting fact that has emerged from our understanding of miRNA biology is the potential use of miRNA mimetics or antagonists as therapeutics. Because miRNA expression is often altered in cancer cells, agents that modulate miRNA activity could potentially produce cancer-specific effects. However, to

Figure 1. miRNAs are transcribed mainly by RNA polymerase II into an immature form of about 80 nucleotides in length called primary miRNAs (pri-miRNA; ref. 3). The stem loop structure of the pri-miRNA is recognized in the nucleus by Drosha and its partner DGCR8 and it is further processed to the precursor miRNA (pre-miRNA; ref. 3). The hairpin-shaped pre-miRNA is then transported from the nucleus to the cytoplasm by exportin-5 (XPO5), where it is loaded by the Dicer–TRBP complex and cleaved into a double-stranded miRNA in a process known as dicing (3). After strand separation, the mature miRNAs, in combination with Argonaute proteins, form the RNA-induced silencing complex (RISC; ref. 4). The expression of the target mRNAs is silenced by miRNAs on the RISC complex, either by mRNA cleavage or by translational repression (4).
evaluate the potential of small RNAs in cancer therapy, an appreciation of how extensively they are tied to the normal cell's gene regulation networks is essential. Overexpression or inhibition of miRNAs can be achieved in several ways. Synthetic miRNA mimetics include siRNA-like oligoribonucleotide duplex and chemically modified oligoribonucleotide (12, 13). Conversely, miRNAs can be inhibited by various modified antisense oligonucleotides, such as 2'-O-methyl antisense oligonucleotide and antagonists. As the first successful tool for knockdown of a miRNA in vivo, antagonists are of special interest because they appear to be delivered to all tissues (except brain) after tail vein injections into mice (14, 15). Delivery of tumor-suppressive miRNAs and silencing oncogenic miRNAs with antagonists have been successful in several mouse models, and this work has progressed to delivery of miRNA-based molecules intranasally, intratumorally, or systemically. Nonetheless, the therapeutic value of miRNA mimetics and antagonists would be greatly enhanced by technical improvement for selective tumor-specific or tissue-specific delivery. In the same regard, miRNAs are also being evaluated for their ability to sensitize cancers to chemotherapy.

Clinical–Translational Advances

Therapies that inhibit oncogenic miRNA function

Despite the overall downregulation of miRNAs observed in human cancer, some specific upregulated miRNAs with oncogenic potential (oncomiRs) are potential therapeutic targets for inhibition. Oncogenic miRNAs can be inhibited by using antisense oligonucleotides, antagonists, sponges, or locked nucleic acid (LNA) constructs (16). The effectiveness of these antisense oligonucleotides has shown promising results in certain cases.

One such case was described in a breast cancer mouse model where the systemic delivery of antagonist-miR-10b to tumor-bearing mice reduced miR-10b levels and prevented the onset of metastasis, suppressing dissemination to the lungs (17). This work suggests that antagonist-miR-10b may prevent metastasis in highly invasive cancers containing elevated miR-10b levels.

Likewise, the use of LNA constructs has shown great success in the treatment of hepatitis C in nonhuman primates, inhibiting the virus replication through inhibition of miR-122 using an LNA construct systemic delivered with no toxicity observed (18). The inhibition of miR-122 in patients who are hepatitis C–positive may decrease their risk of developing hepatocellular carcinoma.

However, cancer cells often exhibit dysregulation of multiple miRNAs; therefore, silencing of a single miRNA might not be sufficient for use in cancers where more than one oncomir is overexpressed. Recent research suggests that several miRNAs can be simultaneously inhibited using one antisense oligonucleotide that targets multiple miRNAs: multiple-target anti-miRNA antisense oligodeoxyribonucleotide—MTg-AMO (19). The authors have described an MTg-AMO that is able to inhibit at the same time 3 very well-described oncomirs that are often overexpressed in many tumors: miR-21, miR-155, and miR-17-5p (19). The use of this MTg-AMO resulted in an enhanced suppression of cancer growth compared with individual inhibition of the miRNAs (19). This technique will allow for simultaneous inhibition of several miRNAs involved in various aspects of cancer biology like angiogenesis, invasion, or metastasis.

The success of miRNA inhibition for therapy will depend on their pharmacokinetic behavior, as this will serve as a basis for predicting how the compound will behave in humans. Most anti-miRs distribute broadly but tend to accumulate in a characteristic pattern with most in the liver and kidney, and each chemical modification alters this characteristic pattern. Thus, it becomes important to characterize each one of the molecules used in animal models for prediction in humans. Also plasma anti-miRs are cleared from plasma within hours by uptake into tissues but, once inside cells, the anti-miRs are very metabolically stable so their clearance is slow and half-lives in tissues are often in the order of weeks, providing therapeutic benefit long after blood levels are near zero (20, 21).

Therapies that restore tumor-suppressive miRNA function

The term "miRNA replacement therapy" has been strategically used to name the treatment that aims to restore miRNAs with tumor-suppressive functions. MiRNA let-7 is one of the best-described tumor-suppressive miRNAs (22). In xenograft models, tumor burden was reduced by intratumoral delivery of let-7b, as well as the burden of KrasG12D/+ lung tumors through the intranasal delivery of let-7a using lentivirus or by the systemic delivery of let-7b (23, 24). Let-7 miRNA is downregulated in multiple cancers; hence, their oncogenic targets are overexpressed, enhancing tumor proliferation, invasion, and metastasis, which makes this cancers perfect targets for the use of let-7 miRNA mimetics as therapeutic tools.

Oncogenic KRAS is targeted by several different miRNAs, but it also inhibits the transcription of a miRNA cluster containing miR-143 and miR-145, through the activation of RAS-responsive element–binding protein 1 (RREB1; refs. 25–27). In a very sophisticated mechanism miR-143 and miR-145 target RREB1 and KRAS, respectively, inhibiting their translation (26). Systemic intravenous delivery of miR-143 and miR-145 to subcutaneous and orthotopic xenografts downregulated RREB1 and KRAS levels (28). A high throughput analysis of 744 cancer samples has revealed miR-143–145 cluster as frequently deleted. Therefore, these tumors, as well as tumors with KRAS overexpression, would be good candidates for therapy using miR-143 and miR-145 miRNA restoration therapy.

p53 protein has been found to affect miRNA processing by enhancing the maturation of several growth-suppressive miRNAs—its third known antitumor activity (29). Most cancerous p53 mutations affect the domain that interferes with miRNA processing and thus may abolish all tumor-suppressor functions. One of the best-studied miRNAs with processing enhanced by p53 that is downregulated in various cancers is miR-34, which stimulates apoptosis or cellular senescence, induces G1 arrest, and prevents
migration (30). Recent research has shown that miR-34 replacement therapy would be of great therapeutic benefit. Delivery of miR-34 mimic either intratumorally or systemically impaired tumorigenesis on a xenograft model of non–small cell lung cancer; in the same regard, systemic delivery of miR-34 reduced tumor growth of KrasLSL-G12D\(^+\) lung tumors (24, 31). Because we know that a high percentage of prostate as well as pancreatic cancers contain p53 mutations and/or attenuated miR-34 expression, miR-34 also should be considered as a good therapeutic approach for these types of cancers (28, 31).

A recent study reports the restoration of miR-26a expression in a hepatocellular carcinoma mouse model (32). The treatment has resulted in suppression of proliferation and induction of apoptosis, inhibiting cancer progression (32). In this model, an adenoviral vector was used to systemically deliver the miR-26a mimic, which can be very limiting due to the challenges it presents. The use of these molecules as therapeutics would undoubtedly have an impact on cancer treatments by allowing the restoration of miRNAs that in most cases target oncogenic transcription factors that are difficult to inhibit through traditional medicinal chemistry (33). Also, miRNA-mimetics present the same pharmacokinetic characteristics described for anti-miRs, which renders them good candidates for use in humans (20, 21).

Despite the promising therapies that aim to restore the function of one miRNA, we are still far from restoring the "normal" expression of miRNAs in tumors. A large body of evidence shows that human tumors are characterized by impaired miRNA processing that leads to global miRNA downregulation. Some chemical compounds alter the expression of a group of miRNAs; therefore, it may be possible to screen for drugs that could shift the miRNA expression profile of a cancer cell toward that of a normal tissue (34). Recent findings have shown that restoring the global miRNA expression (miRNAome) could have a therapeutic effect (35, 36). By modulating multiple miRNAs simultaneously, such a miRNAome modifying approach may be much more effective for therapy than strategies that aim to regulate a single miRNA. Reconstitution of downregulated miRNAs offers the theoretical edge of correcting the malignant defect by inducing small changes in miRNA gene dosage to a homeostatic level, achieving phenotypic alterations that counteract malignant transformation. Few studies of fluoroquinolones have shown a significant growth inhibition of some tumor cells, including transplanted cell carcinoma of the bladder, colorectal carcinoma, and prostate cancer cells (37–39). Enoxacin belongs to this family of synthetic antibacterial compounds based on a fluoroquinolone skeleton (40). Enoxacin has been used to treat bacterial infections ranging from gonorrhea to urinary tract infections (41). Clinically, side effects have been minimal in adults (41). This small molecule also enhances RNA interference (RNAi) induced by either short hairpin RNAs or siRNA duplexes (36). A new "miRNAome-based" strategy to restore global miRNA expression has been suggested (35). The small-molecule enoxacin enhances RNAi and promotes processing by binding to TRBP (35, 36). After being administered to human cancer cells lines and systemically delivered to xenograft mouse models, enoxacin has restored downregulated miRNAs to a more "normal-like" miRNA expression pattern and tumor growth has been inhibited with great efficiency (35). Most importantly, the drug showed no effect on normal cells and was not associated with toxicity in the mouse models (36).

**Using miRNAs to sensitize tumors to chemotherapy**

The design of cancer chemotherapy has become increasingly sophisticated, but there is still no cancer treatment that is 100% effective against disseminated cancer. Chemotherapy resistance occurs when cancers that have been responding to a therapy suddenly begin to grow. Acquired resistance to chemotherapy is a major obstacle to successful cancer treatment. The identification of new approaches that could circumvent this problem would be key to sensitize resistant cells to commonly used cancer therapies. Because miRNAs target a multitude of miRNAs involved in different signaling pathways that are often impaired in cancer, the potential of using miRNAs or antagonists to make the tumors responsive to chemotherapy is of great promise. However, this is still a growing field, and therefore in vitro data are not abundant. Nonetheless, recent in vitro experiments have shed light on what can become the new era of tumor sensitization drugs. One of the most common reasons for acquisition of resistance to a broad range of anticancer drugs is expression of transporters that detect and eject anticancer drugs from cells.

The miRNA miR-9 has been described in a recent report to be a negative regulator of SOX2, which indirectly induces ABCG1 and ABCG6 transporters (42). Therefore, when SOX2 is overexpressed, cancer cells are able to excrete drugs to the extracellular environment becoming resistant (42). The overexpression of mir-9 in a glioma stem cell line (chemotherapy-resistant) resulted in reduced ABC transporter expression and increased drug retention (42). These data provide hope that cancer cell drug reflux can be reduced through manipulation of miRNAs.

Another example is resistance to tamoxifen, a widely used estrogen receptor modulator. These tumors repress miRNAs miR-15 and miR-16 and are able to restore the antiapoptotic BCL-2 expression (43). In this regard, reexpression of miR-15 and miR-16 decreased BCL-2 expression and cells became sensitized to tamoxifen. The same results proved true for miR-342 which forced overexpression sensitized cells to tamoxifen-induced apoptosis (44).

5-Fluorouracil (5-FU) has been used as a chemotherapy agent against cancer for more than 40 years. It acts mainly as a thymidylate synthase inhibitor blocking the synthesis of thymidine and thereby rapidly driving cancer cells to undergo cell death (45). A recent miRNA profiling report has revealed that high miR-21 levels are correlated with poor treatment outcome in colon cancer due to the direct downregulation by miR-21 of genes of the mismatch repair machinery of the cells (46). The same results stand true for hepatocellular carcinoma and pancreatic cancer (47, 48). In this regard, antagonism against miR-21 was able to sensitize...
cultured cells to 5-FU treatment, suggesting this miRNA therapy as a promising path for tumors resistant to 5-FU that overexpress miR-21 (48).

**Off-target effects**

Non-specific side effects are as important as effectiveness and duration of miRNA expression or inhibition. Each miRNA can target up to 200 transcripts directly or indirectly, and multiple miRNAs can target a given gene. Therefore, the potential regulatory network afforded by miRNAs is extremely complex, and the therapeutic outcome of a miRNA therapy will directly depend on the number of targets and the affinities for each one of those targets that are expressed in that specific cellular context. Also, delivery to the appropriate cell type or tissue is an important aspect of effective miRNA mimicry to prevent unwanted side effects.

Some investigators have argued that miRNA mimetics or inhibitors are specific enough to distinguish between similar miRNAs (49, 50). However, cross-reactivity between miRNAs of similar sequence is likely to be unavoidable at high doses of antagonists or agonists. Another possible side effect is that high expression of miRNA mimetics may interfere with endogenous miRNA action by saturating the cellular machinery for miRNA processing or action. This may result in a change in expression of other miRNAs, leading to a deleterious effect in the cells. Indeed, a fatal side effect as a result of saturation of miRNA pathways has been reported (51). To minimize undesirable side effects, the expression or knockdown of a miRNA should be improved so that it is more accurate and controllable. An alternate approach to improving specificity is to target the pre-miRNAs with antisense or siRNA strategies.

Despite all of the progress that has been made in the new era of miRNA therapeutics there is still a significant gap between basic research on miRNAs and clinical application. Extensive preclinical and translational research is required to increase the efficacy and minimize the side effects of miRNA-based therapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

**Conception and design:** S.A. Melo, R. Kalluri

**Writing, review, and/or revision of the manuscript:** S.A. Melo, R. Kalluri

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** S.A. Melo

**Study supervision:** S.A. Melo, R. Kalluri

Received March 22, 2012; revised May 23, 2012; accepted May 25, 2012; published OnlineFirst June 18, 2012.

**References**


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