Review

Cancer Research

The Promise of MicroRNA Replacement Therapy

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Abstract

MicroRNAs (miRNA), a class of natural RNA-interfering agents, have recently been identified as attractive targets for therapeutic intervention. The rationale for developing miRNA therapeutics is based on the premise that aberrantly expressed miRNAs play key roles in the development of human disease, and that correcting these miRNA deficiencies by either antagonizing or restoring miRNA function may provide a therapeutic benefit. Although miRNA antagonists are conceptually similar to other inhibitory therapies, restoring the function of a miRNA by miRNA replacement is a less well characterized approach. Here, we discuss the specific properties of miRNA replacement and review recent examples that explored the therapeutic delivery of miRNA mimics in animal models of cancer. *Cancer Res; 70(18); 7027–30. ©2010 AACR.*

MicroRNAs (miRNA) are small, noncoding single-stranded RNAs that comprise a new class of gene regulators (1). They are highly conserved from plants to humans and are encoded by their respective genes. miRNAs are transcribed from the genome as longer precursor molecules that are cleaved by the nuclear ribonuclease Drosha into approximately 70- to 100-nt-long oligonucleotides that form a distinct hairpin structure. Following nuclear export, this precursor is further cleaved by the RNAse Dicer, which yields a 17- to 25-nt double-stranded oligonucleotide that enters the RNA-induced silencing complex (RISC), a multiprotein complex that separates the mature strand from the passenger strand and facilitates the interaction of mRNAs with sequences that are complementary to the mature miRNA. RISC loaded with miRNA and the target mRNA inhibits the translation of the mRNA by either a silencing mechanism or by degradation of the mRNA. In most cases, the miRNA and mRNA sequences are merely partially complementary, which enables miRNAs to target a broad, but nevertheless a specific, set of mRNAs. To date, more than 900 human miRNA sequences have been annotated and may regulate at least 20 to 30% of all protein-encoding genes.

The discovery of miRNAs adds another layer of gene regulation that is subject to change in human disease, including cancer. Similar to protein-encoding genes, miRNAs are now supported by expression data and experimental evidence *in vitro* and *in vivo* that mark these interesting RNA molecules as promising therapeutic targets: miRNAs frequently acquire a gain or a loss of function in cancer; and miRNAs play a causative role in the development of cancer (2, 3). Aberrant regulation of miRNAs is manifested by differential expression in the tumor tissue relative to the normal adjacent tissue and can be the consequence of genomic rearrangements or altered methylation status of their respective promoter regions. Somatic point mutations, albeit not thoroughly studied, may be another mechanism that leads to the deregulation of miRNAs. Altered expression of miRNAs is apparent in virtually all tumor types and includes blood-borne malignancies as well as solid tumors. The functional consequence of miRNA deregulation became evident as the introduction or repression of a single miRNA can effectively contribute to tumorigenesis or tumor progression. Numerous functional studies using cultured cancer cells and mouse models of cancer have identified miRNAs that function as conventional tumor suppressors or oncogenes. Examples of miRNAs with oncogenic activity are miR-155 and miR-17–92; in contrast, miR-15a, miR-16, as well as miRNAs of the miR-34 and *let-7* families, are tumor-suppressor miRNAs (refs. 2, 4–9 and references therein). The tumor-suppressive or oncogenic activity for many of these miRNAs is not limited to a particular tumor type, in agreement with the supposition that conventional cancer genes function as such regardless of tissue origin. The deregulation of some of these miRNAs also correlates with tumor differentiation status, disease stage, and patient outcome, further suggesting that aberrant miRNA function has a direct impact on tumor development. For instance, low *let-7* levels and high miR-155 levels are indicative of poor survival of patients with non–small cell lung cancer (10). Other miRNAs have specifically been implicated in early tumorigenesis or metastasis, representing unique opportunities for therapeutic intervention that will depend on the context and requirement of therapy.

The therapeutic application of miRNAs involves two strategies. One strategy is directed toward a gain of function and aims to inhibit oncogenic miRNAs by using miRNA antagonists, such as anti-miRs, locked-nucleic acids (LNA), or antagomiRs. These miRNA antagonists are oligonucleotides with sequences complementary to the endogenous miRNA. They carry chemical modifications that enhance the affinity for the target miRNA and trap the endogenous miRNA in a configuration that is unable to be processed by RISC, or

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doi: 10.1158/0008-5472.CAN-10-2010

alternatively, leads to degradation of the endogenous miRNA. Small molecule inhibitors specific for certain miRNAs are also being developed to inhibit miRNA function. The second strategy, miRNA replacement, involves the reintroduction of a tumor-suppressor miRNA mimic to restore a loss of function (Fig. 1). Although the inhibitory approach is more commonly accepted and conceptually follows rules that also apply to small molecule inhibitors and short interfering RNAs (siRNA), miRNA replacement represents a new opportunity to explore the therapeutic potential of tumor suppressors (4, 7, 8, 11). Therapeutically restoring the levels of tumor suppressors in tumor tissues has been investigated in the past by gene therapy; however, a practical application of this approach is still pending. Because the definition of tumor suppressors was restricted to protein-encoding genes, gene therapy usually involves the delivery of a relatively large DNA plasmid or viral vector that encodes the desired protein. Often, vector size, inefficient delivery to target tissues, and the requirement for nuclear localization represent technical challenges that limit this approach to local, rather than systemic, administration (12, 13). Thus, despite a strong scientific rationale for cancer treatment, logistic obstacles associated with gene therapy leave the full therapeutic benefit of using tumor suppressors unanswered. miRNAs provide a new opportunity because, unlike proteins, miRNA mimics are substantially smaller, will merely have to enter the cytoplasm of target cells to be active, and can be delivered systemically using modes and technologies that are also used for siRNAs. Therefore, the delivery hurdle for miRNA mimics seems to be less an impediment than it is for protein-encoding DNA. In addition, several other key observations support the concept of miRNA replacement therapy: (i) the majority of differentially expressed miRNAs is suppressed in tumor tissue relative to normal tissues, indicating that the probability for miRNAs as tumor suppressors is greater than the probability as oncogenes (14); and (ii) inhibition of endogenous miRNA processing induces oncogenic transformation and augments tumorigenesis, suggesting that the tumor suppressive role of miRNAs prevails over an oncogenic role (15). Another advantage of miRNA mimics is the fact that a miRNA mimic has the same sequence as the depleted, naturally occurring miRNA and, therefore, is expected to target the same set of mRNAs that is also regulated by the natural miRNA. Nonspecific off-target effects are unlikely as miRNA mimics are expected to behave like the natural counterpart for which the proper miRNA-mRNA interactions have evolved over a billion years. The strongest rationale for exploring the therapeutic potential of miRNAs, however, is based on the observation that a single miRNA can regulate multiple oncogenes and oncogenic pathways that are commonly deregulated in cancer (3). Therefore, miRNAs act in accordance with our current understanding of cancer as a "pathway disease" that presumably can only be successfully treated when intervening with multiple oncogenic pathways (16). The inhibitory effects induced by miRNAs on any particular target may be mild and may merely lead to a subtle reduction of protein expression; however, the simultaneous down-regulation of a broad set of targets has far-reaching biological consequences that determine the course of the cellular phenotype. The rapid and coordinated manipulation of protein levels across multiple pathways endows these regulatory RNAs with the ability to instantly switch between cellular programs. By restoring the expression of tumor suppressive miRNAs, miRNA replacement therapy seeks to reinstate those cellular programs that are active in normal cells and interfere with oncogenic programs necessary for the malignant phenotype.

To date, few tumor suppressor miRNAs have been discovered for which the proof of concept of miRNA replacement therapy has been shown in preclinical animal models of cancer. The concept of miRNA replacement therapy is perhaps best exemplified by the *let-7* miRNA that was originally identified as a switch gene required for proper development in *Caenorhabditis elegans* (3). *let-7* is expressed at reduced levels in non–small cell lung cancer relative to normal lung tissue, which inversely correlates with the expression of the RAS

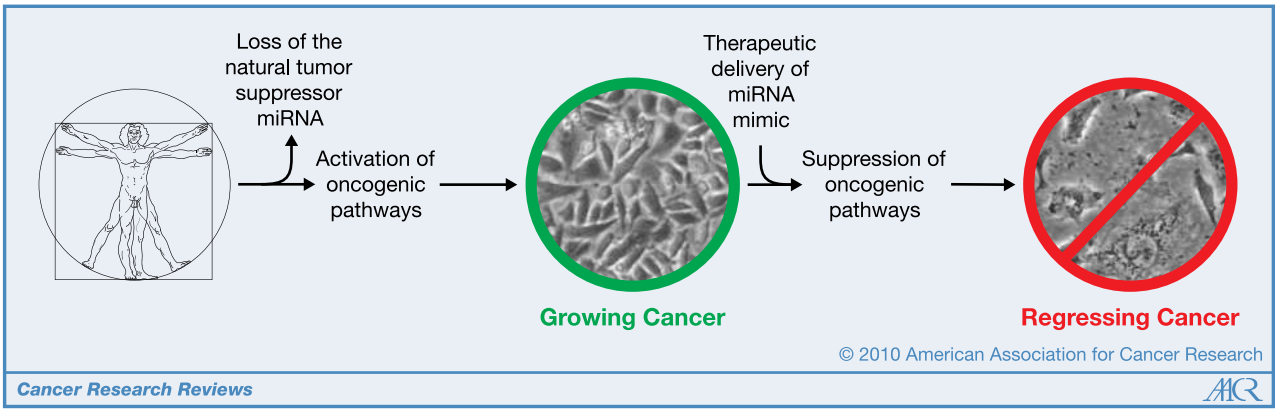

Figure 1. Oncology-directed miRNA replacement therapy. Loss of a tumor suppressor miRNA leads to hyperactivation of inherently oncogenic pathways and tumorigenesis. Administration of a miRNA mimic reinstates the function of the missing tumor suppressor miRNA and suppresses oncogenic pathways and cancer cell growth.

oncoprotein, a key cancer gene frequently activated in this type of cancer (5). Inverse expression of *let-7* and RAS was explained by the fact that *let-7* directly suppresses members of the RAS oncogene family and that a loss of *let-7* function leads to an increase in RAS protein levels (5). Other tumor-promoting genes directly suppressed by *let-7* include HMGA2, Myc, cyclin D, CDK6, and CDC25A; and hence, a decrease of *let-7* sets free a number of oncogenic factors. Functional studies using cultured lung cancer cells, as well as mouse models of lung cancer, showed that the reintroduction of *let-7* mimics blocks the proliferation of cancer cells and reduces growth of existing lung tumors in the animal (4, 6–8). Similarly, specific inhibition of *let-7* augmented tumorigenesis in a KRAS-induced mouse model, further indicating that *let-7* functions as a *bona fide* tumor suppressor, and that replacing the missing miRNA by a *let-7* mimic provides a therapeutic benefit (8).

Another example that shows the value of miRNA replacement is provided by miR-34a (17). In this case, a synthetic miR-34a mimic was delivered systemically to lung tumors in mice, which showed reduced levels of endogenous miR-34a. Therapeutic delivery of miR-34a led to an accumulation of miR-34a in the tumor tissue, suppression of known miR-34a target genes, and, most importantly, inhibition of lung tumor growth. The miR-34a mimic was delivered in a lipid-containing formulation that did not induce a nonspecific immune response, which has been implicated previously to be a contributor of therapeutic effects in other oligonucleotide-containing formulations. Hence, the study suggests that the miR-34a mimic was successfully delivered into tumor cells and that the anti-oncogenic activity of miR-34a is a specific result of its mechanism of action. The rationale for using miR-34a as a tumor suppressive agent is based on expression and functional data showing that miR-34a is lost or expressed at reduced levels in most human cancer types, including lung cancer, and that the reintroduction of miR-34a into cultured cancer cells blocks cell-cycle progression and induces apoptosis as well as senescence pathways (9). The tumor suppressor function of miR-34a is further highlighted by the fact that it is a transcriptional target of TP53 (p53) and, presumably, a fundamental component in the p53 tumor suppressor pathway (9). Other miRNAs, for which the concept of miRNA replacement therapy has been shown in mouse models of cancer, include miR-16 and miR-26a (11, 18).

Attacking multiple genes relevant to human disease at once is viewed as a powerful ability of therapeutic miRNA mimics. However, it also raises concerns about potential toxicity in normal tissues, especially under conditions in which the therapeutic delivery of miRNA mimics will also lead to an accumulation of exogenous miRNA in normal cells. These toxic effects might be the result of overloading RISC with the exogenous miRNA, thereby competing with endogenous miRNAs necessary for normal cellular welfare, and/or hyperactivating cellular pathways that will also reduce the viability of normal cells. Although these suppositions are well founded, *in vivo* evidence for toxicity induced by miRNA mimics is still lacking. Mouse studies that evaluated the therapeutic delivery of tumor suppressor miRNAs failed to reveal adverse events associated with the miRNA and suggest that delivery of miRNA to normal tissues was well tolerated (7, 11, 17, 18). The miRNA sequences used in these studies are identical between the mouse and human species, and, therefore, differences in homology will not account for the lack of toxicity in normal tissues. Thus, several hypotheses begin to form that might explain these observations, none of which has been investigated in detail, and some of which are purely speculative: Therapeutic miRNA mimics may be better tolerated by normal cells than cancer cells because (i) pathways activated or repressed by the miRNA mimic are already activated or repressed by the endogenous miRNA; (ii) administration of therapeutic miRNA mimics is only an insignificant incremental increase of what is already present in normal cells; (iii) normal cells are not addicted to oncogenic pathways and manage to recover from the therapeutic dose used; or (iv) normal cells have the ability to regulate the activity or presence of the miRNA mimic, whereas cancer cells do not. As therapeutic programs advance miRNAs closer to the clinic, it will become critical to study miRNA-induced effects in normal cells and to assess potential toxicity in higher species.

Taken together, miRNA replacement has emerged as a highly promising therapeutic strategy. It encompasses several conceptual aspects of traditional gene therapy and technical features of siRNA therapeutics. However, given the fundamental differences in the approach, mechanism of action, and outcome, miRNA mimics should be viewed as a new class of therapeutics. Available data, showing that miRNAs can function as *bona fide* tumor suppressors and that synthetic versions of these miRNAs robustly interfere with tumor growth in animal models, strongly support the development of miRNA mimics. In addition, recent data implicating miRNAs in self-renewing tumor-initiating cancer cells (cancer stem cells) may significantly broaden the scope of miRNA mimics and may suggest that miRNAs can become valuable tools to eliminate cancer cells frequently associated with chemoresistance, metastasis, and recurrence (19, 20). The main challenge for successful translation into the clinic remains *in vivo* delivery, which will be the focus of future therapeutic development efforts to harness the full potential of miRNAs.

Disclosure of Potential Conflicts of Interest

A.B.G. and D.B. are employees of Mirna Therapeutics, Inc., which develops miRNA-based therapeutics. M.W. is an employee of Asuragen, Inc., and founder of Mirna Therapeutics, Inc.

Acknowledgments

We thank Drs. Paul Lammers and Sylvie Beaudenon for critical reading of the manuscript. We apologize to the many authors whose work could not be quoted due to lack of space.

Grant Support

Grant from the National Cancer Institute (A.G. Bader, 1R43CA134071).

Received 06/03/2010; accepted 07/14/2010; published OnlineFirst 08/31/2010.

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