MicroRNA therapeutics: a new niche for antisense nucleic acids

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MicroRNA molecules (miRNAs) are naturally occurring triggers of the RNA-interference pathway. The first identified miRNA, lin-4, was discovered in Caenorhabditis elegans >20 years ago. What began as a curiosity in this model organism has expanded into almost every area of biology; there are now 326 confirmed miRNA genes in humans and the total is predicted to reach 1000. Each miRNA has the potential to regulate hundreds of mRNAs; therefore, there are likely to be few biological pathways not impacted by miRNA regulation. Recent evidence has suggested that miRNAs might be viable therapeutic targets for a wide range of diseases, including cancer. A recent article by Stoffel and colleagues has demonstrated remarkably effective inhibition of miRNAs in vivo, thus providing an entry point into the promising new arena of miRNA therapeutics.

MicroRNA as regulators of gene expression

MicroRNA molecules (miRNAs) are short, non-coding RNAs that regulate gene expression by controlling the efficiency of mRNA translation [1]. They are transcribed as long primary transcripts that are capped, polyadenylated and spliced, and thus resemble conventional mRNAs (Figure 1). However, unlike mRNA, the active region of the miRNA is contained within a ~70-nucleotide hairpin structure that forms in the RNA transcript. This hairpin structure is cleaved by the endonuclease enzymes Drosha and Dicer to yield a ~21-nucleotide double-stranded RNA (dsRNA) [2]. This RNA product, which is referred to as the ‘mature’ miRNA, resembles a small-interfering RNA (siRNA) and is assembled into the RNA interference (RNAi)-effector complex RISC (RNA-induced silencing complex). The miRNA directs RISC to partially complementary mRNA sites by base-pairing. Consequently, the expression of targeted mRNAs is blocked either by translational repression or by mRNA degradation. In this way, a cell can use the RNAi pathway to regulate the expression of many genes simply by transcribing the appropriate miRNA. Because miRNAs do not encode proteins, they are not traditional therapeutic targets of small-molecule inhibitors. Thus, the development of miRNA inhibitors presents unique challenges for researchers.

MicroRNA function in human biology and disease

The most extensively characterized miRNAs are the first to be discovered, lin-4 and let-7 of Caenorhabditis elegans. These miRNAs have essential roles in controlling timing events during larval development. Although the biological roles of most mammalian miRNAs are still unknown, several recent articles have provided a glimpse of what these non-coding RNAs might be doing. The tissue-restricted expression of many miRNAs suggests that they might be involved in the determination or maintenance of cell lineage [3]. Such a role has been demonstrated for miR-181 in B-cell-progenitor determination [3]. Furthermore, regulation of the homeobox transcription factor HoxB8 by miR-196 indicates that miRNAs have a role in development [3]. Several reports have demonstrated a role for miR-1 in the development of the heart and skeletal muscle. miRNAs have also been linked to the regulation of insulin secretion [3].

From these few examples, it is already clear that miRNAs are important for diverse cellular processes. Consequently, dysregulation of miRNA function might lead to human disease, and several examples support this hypothesis. Tourette's syndrome, a neurological disorder with an unknown etiology, might result from mistargeting of a miRNA if one of its targets has been mutated [4]. A single nucleotide polymorphism (SNP) that correlates with this disease is located in the 3’ untranslated region (3’ UTR) of the gene SLITRK1 (SLIT and TRK-like 1); this SNP resides within the binding site for miR-189, rendering SLITRK1 a better target of the miRNA. Cancer has also been linked to aberrant miRNA expression. A cluster of miRNAs, miR-17-92, is overexpressed in some lymphoma and solid tumors. Ectopic expression of these miRNAs in a mouse model for Burkitt's lymphoma led to accelerated and disseminated disease [5]. Interestingly, miR-17-92-induced tumors displayed reduced apoptosis. A connection between miRNAs and apoptosis has been previously established in Drosophila [1]. In contrast to such oncogenic miRNAs, the reduced expression of many other miRNAs in primary tumor samples suggests that these miRNAs might act as tumor suppressors [6]; most notable are the human homologs of C. elegans let-7. These miRNAs are predicted to target the oncogene RAS, leading to growth suppression [7], and their downregulation in tumors might be an important step in the development of cancer.
Antisense inhibitors of miRNA action

The widespread role of miRNAs in biology makes them valuable targets for therapeutic intervention. Conventional therapeutic targets are proteins that interact with other proteins or with small molecules: such ‘druggable’ targets are susceptible to inhibitors that disrupt their functional interaction. For example, the anti-ulcer drug Tagamet (cimetidine) inhibits the interaction between histamine and its H₂ receptors on the parietal cells of the stomach. Although miRNAs do not encode proteins, they have significant potential as therapeutic targets. The base-pair interaction between miRNAs and mRNAs is essential for the function of miRNAs. Therefore, the most logical inhibitor of such interactions is a nucleic acid that is antisense to the miRNA and base-pairs with the miRNA in competition with cellular mRNAs. This hypothesis was tested directly in cultured cells by two independent research groups [8,9]: they transiently transfected 2'-O-methyl-modified antisense RNAs into several independent miRNAs and showed specific inhibition. The 2'-ribose modification of the RNA backbone prevented nuclease

Figure 1. Steps in the biogenesis of microRNA molecules (miRNAs) and points of intervention by miRNA antisense inhibitors. miRNAs are generated as primary transcripts by RNA polymerase II and, after being processed by the endonuclease Drosha, the stem–loop structure of the precursor miRNA (pre-miRNA) is exported out of the nucleus. The endonuclease Dicer releases the ~22-nucleotide mature miRNA, which is then assembled into the RNA-induced silencing complex (RISC). The miRNA directs RISC to partially complementary mRNA sites, resulting in inhibition of translation (right). Nucleic acids that are antisense to the mature miRNA sequence (e.g. the ‘antagomirs’ of Stoffel and colleagues [11]) can duplex with the miRNA and, therefore, can function as competitive inhibitors of miRNA binding to its target mRNAs (red arrow). This results in re-expression of targets (left).

Antagomir-122


Mature miR-122

Figure 2. Structure of the miRNA inhibitor antagomir-122. The sequence of miR-122 is shown for comparison, in the orientation in which it would base-pair with its antagonist, antagomir-122. Black letters indicate standard RNA bases, and red letters indicate 2’-O-methyl-modified bases. Black dashes indicate phosphodiester linkage, and blue dashes indicate phosphorothioate linkage. Abbreviation: Chol, cholesteryl moiety.
degradation in the culture media and, importantly, also prevented endonucleolytic cleavage by the RISC nuclease, leading to irreversible inhibition of the miRNA.

In vivo inhibition of miRNAs

Although the studies in cultured cells were promising, the key development was modifying the miRNA inhibitors for in vivo use. Long-term research efforts in antisense therapies have led to effective strategies for the pharmacological delivery of nucleic acids; this has accelerated the development of siRNA therapeutics, and now, also miRNA therapeutics [10,11]. Stoffel and colleagues [11] have recently demonstrated in vivo inhibition of four miRNAs by modified antisense RNAs, which they term ‘antagomirs’. Their strategy involves three types of modification (Figure 2). The RNA backbone is modified, at each nucleotide, by an O-methyl moiety at the 2′-ribose position. The terminal nucleotides at both ends are also modified with a phosphorothioate linkage, in contrast to the standard phosphodiester linkage in RNA and DNA. These modifications provide nuclease resistance, which is essential for a nucleic acid that will be exposed to abundant serum and cellular nucleases. The third modification is a cholesteryl functionality at the 3′ end of the nucleic acid. This improves pharmacokinetic properties: it increases binding to serum proteins (improving stability and half-life in serum) and it enhances cellular uptake [12].

Stoffel and colleagues injected antagomirs into adult mice and showed remarkably effective inhibition of miRNA. Surprisingly, the targeted miRNAs were not only blocked but also degraded by an unknown mechanism. Four antagomirs, which targeted distinct miRNAs, were successfully tested. Targeting was effective in all tissues except the brain, presumably because of restricted diffusion of the charged nucleic acid across the blood–brain barrier.

Targeting of the liver-specific miRNA miR-122 upregulated several hundred miRNAs, many of which contained putative binding sites for the miRNA. Plasma cholesterol levels were decreased by 40% using this treatment, which is consistent with the hypothesis that liver function was diminished by inhibition of miR-122. This is one example of a therapeutic outcome due to inhibition of miRNA function.

Concluding remarks

The work by Stoffel and colleagues [11] provides an exciting first step towards miRNA therapy. Further progress will depend on a better understanding of miRNA function in mammals. Interestingly, such research can benefit from antagomirs. The ability to inhibit miRNAs with such a simple approach will accelerate research on these non-coding RNAs; for example, it might be possible to screen anticancer antagomirs using established mouse models of cancer. Such data would contribute to basic science research in the field of cancer biology and to identification of potential therapeutic targets. Oncogenic miRNAs, including miR-17–92, are an obvious target, and inhibitors of these miRNAs should be tested against mouse models of cancer.

A drawback to the antisense modifications developed by Stoffel and colleagues [11] is their inability to target the brain; further refinements are needed to address this limitation (Box 1). In addition, the ability to target disease sites selectively might improve the therapeutic value of miRNA inhibitors. Such targeting has been described for siRNAs, and similar approaches might be effective for antagomirs [13].

References

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Box 1. Outstanding questions

- The required intravenous dosage of miRNA is high. What strategies can be employed to reduce dosage to manageable levels? Are other delivery routes possible?
- Inhibition of miRNA has not been observed in the brain. How can this limitation be overcome?
- Is it possible to design antagomirs that target specific tissues or disease sites?

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