Suppressing cancer with miR-34a

By Tim Fulmer, Senior Writer

A team of researchers at **Mirna Therapeutics Inc.** has found that systemically delivered microRNA-34a blocks tumor growth in mouse models of lung cancer.[1] The miRNA targets multiple pro-oncogenic pathways and could potentially show activity against a broad range of cancers. Mirna is optimizing the formulation and hopes to submit an IND in late 2011.

Over the past five years, multiple labs have reported associations between cancer cells and altered expression profiles of miRNA. Compared with healthy tissue, some tumors show overexpression of miRNAs, whereas other tumors show abnormal downregulation of one or more miRNAs.[2,3]

A prime example of the latter is miR-34a. Several labs have independently found low levels of miR-34a in a variety of solid tumors in mice and humans, suggesting that miR-34a functions as a tumor suppressor in normal tissue.[4,5] Indeed, overexpression of miR-34a leads to cell cycle arrest and apoptosis of cancer cells *in vitro*.[6–8]

The open question was whether delivery of an miR-34a mimic would treat tumors *in vivo*. The Mirna team, led by Associate Director of Research Andreas Bader, set out to answer that question by focusing on lung cancer. A previous study by Spanish researchers showed that low miR-34a expression correlated with a greater risk of relapse in patients with non–small cell lung cancer (NSCLC).[9]

Bader's group also looked at miR-34a levels in tissue samples from NSCLC patients. They found that 63% of the samples (20 of 32) had lower miR-34a expression than samples from the corresponding normal adjacent lung.

NSCLC cell lines showed similar reductions in miR-34a expression.

The next question was whether reintroducing miR-34a might reverse the oncogenic phenotype *in vitro*. In a panel of cultured lung cancer cells, the team found that miR-34a transfection reduced cell growth compared with scrambled miRNA transfection.

Finally, the researchers looked at whether miR-34a could block tumor growth in animals.

Because naked miRNA-34a rapidly degrades *in vivo*, the first priority was developing a delivery vehicle. To help them with that, the Mirna scientists collaborated with scientists at **Bioo Scientific Corp.** to develop a lipid-based delivery formulation called MaxSuppressor *In Vivo* RNA-LANCEr II.

In mouse lung cancer xenografts, both intratumoral and systemic delivery of the lipid-formulated miR-34a significantly blocked tumor growth compared with delivery of scrambled miRNA ($p<0.01$). The findings were published in *Cancer Research*.

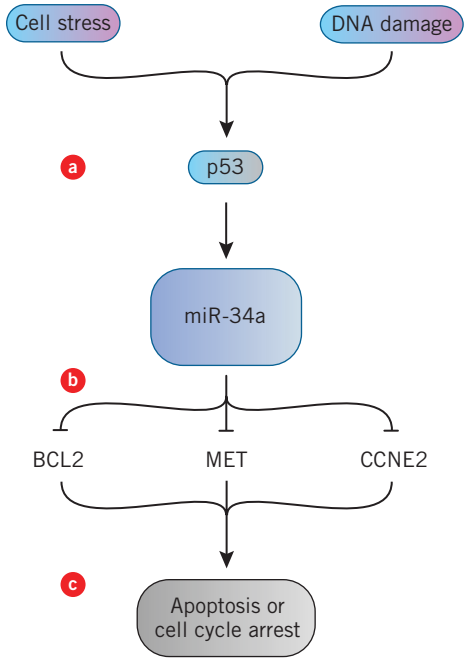

Figure 1. Replenishing miR-34a in cancer. Mirna Therapeutics Inc. has systemically delivered microRNA-34a (miR-34a) to treat non–small cell lung cancer (NSCLC) in mice.

In normal cells, oncogenic signals like stress and DNA damage activate the tumor suppressor tumor protein p53 (TP53; p53) [**a**], which in turn activates miR-34a. Once activated, miR-34a also acts as a tumor suppressor, blocking expression of multiple downstream targets implicated in cancer, including B-cell lymphoma 2 (BCL-2; BCL2), c-Met proto-oncogene (HGFR; MET) and cyclin E2 (CCNE2) [**b**]. The result of miR-34a's action is apoptosis, cell cycle arrest or senescence [**c**].

In cancer, miR-34a is aberrantly downregulated and can no longer act as a tumor suppressor. Thus, the Mirna researchers suggest that delivering exogenous miR-34a to tumor tissue could reactivate the pathway and kill cancer cells.

The power of miR-34a "lies in the fact that it is able to target multiple oncogenic pathways in tumor tissue and could thus show activity against a broad range of cancers," said corresponding author Bader.

When he was a researcher in the lab of Eric Fearon at the **University of Michigan**, Guido Bommer and colleagues reported in 2007 that miR-34a is regulated by the tumor suppressor tumor protein p53 (TP53; p53) and is a key mediator of the downstream effects of p53 *in vitro*.[10] In response to pro-oncogenic cell stress, the activation of p53 triggers the upregulation of miR-34a, which then inhibits its many targets downstream of p53 to cause cell cycle arrest or induce apoptosis (*see* **Figure 1, "Replenishing miR-34a in cancer"**).

Bommer, who is now group leader at the **de Duve Institute**, told *SciBX* that "miR-34a is special in that it is highly conserved across species with regard to its sequence, target genes and mode of regulation. Several of the target genes of miR-34a are either known oncogenes or involved in cellular proliferation, including cyclin E2, CDK6 and BCL2."

"By hitting many targets at once, this approach is consistent with the idea that miRNAs exert their therapeutic effect through the cumulative effect of a large number of small changes," said Alexander Pertsemlidis, assistant professor of internal medicine at **The University of Texas Southwestern Medical Center at Dallas**.

Heiko Hermeking, professor of experimental and molecular pathology at the **Ludwig Maximilian University of Munich**, suggested one approach to employing the target to develop drugs.

"Downregulation of miR-34a is often caused by aberrant methylation of the *miR-34a* gene, which could thus serve as a biomarker of miR-34a deficiency and a method for identifying patients who might respond to miR-34a therapy," he said.

In 2008, Hermeking and colleagues reported that *miR-34a* expression is silenced by aberrant methylation in multiple carcinoma cell lines, including prostate, breast, lung, colon and bladder.[11]

Hermeking told *SciBX* that he has a pending patent (US12/490853) that covers the use of methylation patterns in the *miR-34a* gene promoter as a cancer diagnostic.

Special delivery

With the tumor-suppressive mechanism of miR-34a well established, more work is needed to better characterize the safety and tolerability of systemically delivering the miRNA.

Key studies include determining whether exogenous miR-34a has unwanted effects in normal tissues and whether delivery of the lipid formulation generates an immune response after repeated administration, according to Alexander Nikitin, associate professor of pathology and leader of the Cornell Stem Cell Program at **Cornell University**.

In the *Cancer Research* paper, systemic delivery of formulated miR-34a was not toxic to the liver, kidney and heart of mice, as judged by serum enzyme levels. Nor did a single i.v. dose of the formulation induce an immune response, as measured by serum cytokine levels.

Bommer said longer-term safety studies may be necessary. "While over the short term the compound seems to be well tolerated in mice, it will also be necessary to demonstrate that the long-term use of the lipid-based delivery system is safe," he told *SciBX*.

Bader said the pharmacokinetics of the lipid-based miR-34a formulation are being optimized and that Mirna is investigating other, undisclosed delivery technologies as potential alternatives to the one used in the paper.

Mirna is aiming for an IND submission in late 2011 for miR-34a. According to Bader, the company has "early fundamental patent filings on the use of miR-34a for the treatment of multiple cancer types."

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COMPANIES AND INSTITUTIONS MENTIONED

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