Tumor-suppressing miRNAs

By Tim Fulmer, Senior Writer

A team of U.S. researchers has identified a microRNA that could play a role as a tumor suppressor in hepatocellular carcinoma.¹ The molecule significantly reduced tumor burden in murine liver cancer models, but delivery and specificity hurdles remain before the compound is ready for the clinic.

Over the past five years, multiple labs have found that virtually all cancers are associated with altered miRNA expression profiles compared with those of healthy tissue. Some cancers are linked to overexpression of miRNAs in tumor tissue, whereas other cancers display abnormal downregulation of one or more miRNAs.^{2,3}

Joshua Mendell and colleagues at **The Johns Hopkins University School of Medicine** and **The Ohio State University** have focused on miRNAs that are downregulated in cancer, hypothesizing that such miRNAs might function as tumor suppressors in normal tissue. If so, enhancing levels of those miRNAs could help stop tumor proliferation.

The researchers previously found that multiple miRNAs were downregulated in B cell lymphoma, including miRNA-15a, miRNA-16, miRNA-26a, miRNA-34a and miRNA-195.⁴ To extend the findings to solid tumors, levels of those miRNAs were profiled in a mouse model of liver cancer.

Of the five molecules, miRNA-26a was the most downregulated in the murine tumors compared with normal liver tissue. It also was expressed at significantly lower levels in a panel of human HCC biopsies than in normal liver biopsies ($p < 0.02$). Finally, retroviral overexpression of miRNA-26a in cultured human HCC cells resulted in lower proliferation than treatment with empty retroviral vector.

To test the hypothesis that miRNA-26a functions as a tumor suppressor in liver cancer, the researchers devised an adeno-associated virus (AAV) vector system to deliver miRNA-26a to tumor tissue. In mice with liver tumors, systemic delivery of the miRNA-26a-containing vector, as compared with empty vector, significantly reduced tumor burden and liver-to-body weight ratio ($p < 0.05$ for both).

The findings were published in *Cell*.

Staying on target

Because a single miRNA can potentially target hundreds of different mRNAs, systemic delivery of miRNA-26a could modulate pathways in both tumors and healthy tissue. Thus, a more thorough characterization of miRNA-26a at both the molecular and cellular levels is likely needed to rule out off-target effects.

“Although miRNA-26a triggered apoptosis in mouse tumor tissue, the miRNA also likely influences expression levels of targets in cellular pathways in healthy tissue. Systemic delivery of the miRNA could thus require a narrow dosing window that is toxic to tumor cells and yet doesn’t trigger antiproliferative effects in healthy tissue,” said Aurora Esquela-Kerscher, assistant professor of microbiology and molecular cell biology at the **Eastern Virginia Medical School**.

As a result, she thinks a good next step “would look at a range of miRNA-26a delivery doses to better determine that window.”

Future studies should also analyze miRNA-26a expression data from a larger sample size than the eight HCC tumor biopsies described in the paper, according to Alexander Pertsemlidis, assistant professor of internal medicine at **The University of Texas Southwestern Medical Center at Dallas**.

“That would help determine if loss of miRNA-26a expression is indeed a general feature of advanced hepatocellular carcinoma,” he said. “Although one can hope that increasing miRNA-26a levels will suppress tumorigenesis in all HCC cases, it may be more reasonable

to expect that miRNA-26a therapy will work primarily in those instances where significant loss of miRNA-26a expression occurs.”

Ruling out the possibility that delivering exogenous miRNA-26 could trigger an unwanted immune response will also be important, said Andreas Bader, senior scientist at miRNA biotech **Mirna Therapeutics Inc.**, a subsidiary of **Asuragen Inc.**

“Oligo-based therapeutics have been criticized by some for inducing a nonspecific immune response that could even mimic the effects of an miRNA-targeted therapeutic. Any

characterization of a potential miRNA replacement therapy like the one in the *Cell* paper should therefore probably include at least monitoring cytokine levels during treatment,” said Bader.

Mirna has identified multiple tumor-suppressor miRNAs, and the company is developing mimics of those molecules to treat a variety of cancers. At the Keystone Symposium on MicroRNA and Cancer last month, the company presented data showing that systemic delivery of a lipid-based formulation of miRNA-34 inhibited tumor growth and metastasis in mouse models of lung and prostate cancer without triggering an immune response.

Mendell, a corresponding author on the *Cell* paper and associate professor of pediatrics, molecular biology and genetics at Johns Hop-

“It may be more reasonable to expect that miRNA-26a therapy will work primarily in those instances where significant loss of miRNA-26a expression occurs.”

**—Alexander Pertsemlidis,
The University of Texas
Southwestern Medical
Center at Dallas**

kins, told *SciBX* that a nonspecific immune response is probably not responsible for the tumor reductions his group observed. Instead he noted that miRNA-26a caused cell-cycle arrest and reduced proliferation of human liver cancer cells in culture.

Delivering the goods

Mendell said part of the rationale for choosing miRNA-26a is that the miRNA is normally highly expressed throughout the body. Thus, normal cells may be tolerant of increased miRNA-26a expression caused by uptake of the AAV vector.

Other researchers, however, expressed concern over the use of AAV vectors.

“The *Cell* paper’s approach of using AAV vectors to deliver DNA encoding miRNAs potentially brings with it all the well-known drug development and regulatory challenges associated with gene therapy,” said Peter Linsley, CSO of **Regulus Therapeutics Inc.** “Looking forward, it may be better to adapt the miRNA-26a strategy to some form of encapsulated delivery technology.”

Thus, a fully processed duplex miRNA could be packaged in a liposome or some other type of nanocarrier for systemic delivery, added Scott Hammond, associate professor of cell and developmental biology at **The University of North Carolina at Chapel Hill** and member of Regulus’ scientific advisory board.

Regulus has multiple undisclosed miRNA mimics in preclinical development to treat cancer. Those compounds are delivered systemically using liposomal encapsulation.

Mendell said his lab is looking at multiple approaches to delivering miRNA-26a to tumors, including encapsulation technologies. “None-theless, we also believe AAV vectors have their own advantages,”

he said. “Sustained expression of miRNA is possible with a single delivery of the *miR-26a* gene. Secondly, our work and that of others has shown that AAV vectors are particularly suited for targeting liver cells and allow for long-term gene expression without toxicity.”

Johns Hopkins has applied for patents covering the use of miRNA-26a replacement therapy to treat cancer, said Mendell.

Ongoing work by Mendell and colleagues seeks to identify additional tumorigenic miRNAs implicated in cancer, including those involved in regulation of p53 and c-Myc.

Fulmer, T. *SciBX* 2(26); doi:10.1038/scibx.2009.1031
Published online July 9, 2009

REFERENCES

1. Kota, J. *et al. Cell*; published online June 12, 2009; doi:10.1016/j.cell.2009.04.021
Contact: Joshua Mendell, The Johns Hopkins University School of Medicine, Baltimore, Md.
e-mail: jmendell@jhmi.edu
Contact: Jerry Mendell, Nationwide Children’s Hospital, Columbus, Ohio
e-mail: jerry.mendell@nationwidechildrens.org
2. Calin, G. & Croce, C. *Nat. Rev. Cancer* **6**, 857–866 (2006)
3. Ventura, A. & Jacks, T. *Cell* **136**, 586–591 (2009)
4. Chang, T.-C. *et al. Nat. Genet.* **40**, 43–50 (2008)

COMPANIES AND INSTITUTIONS MENTIONED

Asuragen Inc., Austin, Texas
Eastern Virginia Medical School, Norfolk, Va.
The Johns Hopkins University School of Medicine, Baltimore, Md.
Mirna Therapeutics Inc., Austin, Texas
The Ohio State University, Columbus, Ohio
Regulus Therapeutics Inc., Carlsbad, Calif.
The University of North Carolina at Chapel Hill, Chapel Hill, N.C.
The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas