MicroRNAs As Onco-miRs, Drivers of Cancer

By Karyn Hede

Evidence has been mounting for a decade that small regulatory RNAs called microRNAs (miRNAs) are linked to cancer formation and progression. But miRNAs' role as a driver of cancer has been less certain.

Now animal studies are offering the first tantalizing hints that some miRNAs, known as onco-miRs, can actually set tumor formation in motion and that restoring the function of tumor-suppressing miRNA can eliminate tumors.

Many studies have reported that specific miRNAs, which bind to complementary sites on mRNAs and prevent their translation into proteins, are associated with more invasive cancers. But these findings all showed correlation, not causation.

Carlo Croce, M.D., and his colleagues at the Ohio State University Comprehensive Cancer Center in Columbus provided the first clues that miRNAs could drive oncogenesis when the team inserted the gene encoding miR-155 into fertilized mouse eggs. The researchers reported in 2006 that offspring whose genomes included miR-155 developed enlarged spleens full of immature B cells, a condition that mimicked some forms of leukemia and lymphoma. Offspring lacking the transgenic miR-155 did not have enlarged spleens and developed normally. However, the researchers were unsure how miR-155 could cause disease.

This spring, the same researchers reported in Proceedings of the National Academy of Sciences that, at least in colon cancer cells in the lab, miR-155 can silence crucial DNA mismatch repair genes. When the researchers examined patient tumor samples, they found elevated miR-155 levels in 18 of 83 samples tested, but not in adjacent normal tissue. In all samples where miR-155 levels were three times higher than normal, mismatch repair gene expression was reduced. The researchers suggest that the finding could explain a rare colon cancer syndrome in which mismatch repair genes appear normal but produce no protein.

New Transgenic Mouse

To date, the strongest evidence of a single miRNA causing cancer appeared online in Nature August 8. Frank Slack, Ph.D., and his collaborators, Pedro Medina, Ph.D., and Mona Nolde, Ph.D., at Yale University in New Haven, Conn., report creating a transgenic mouse carrying miR-21, a known onco-miR. Genetic machinery, inserted along with miR-21, allowed the researchers to turn miR-21 expression off or on by changing the animals' diet.

The researchers chose miR-21 on the basis of the findings from Croce's lab that were subsequently validated by findings from the laboratory of Curtis Harris, Ph.D., chief of the National Cancer Institute's Laboratory of Human Carcinogenesis. Harris's lab has found miR-21 elevated in most human cancers. In 2008, the group published a report in the Journal of the American Medical Association showing not only that miR-21 was elevated in colon cancer but that its expression increased with tumor progression.

In Slack's experimental model, when miR-21 expression was turned on, the animals quickly developed a pre-B-cell lymphoid malignancy similar to that demonstrated in Croce's experiments. But unlike the miR-155 researchers, this team could turn off the expression of miR-21. When they did, the animals' tumors completely regressed within a few days. What's more, when these researchers injected cancerous cells from five of the animals into immunocompromised mice, all the mice developed solid tumors in less than 3 weeks, confirming that the cells were malignant and not just blocked in their differentiation. As a control group, two mice received normal spleen cells and did not develop tumors.

"The experiment that Frank did is a beautiful genetic proof that miR-21 is an onco-microRNA, and when it is highly dysregulated it can lead to cancer," said Croce.
Slack’s group is now using the mouse model to explore the molecular targets of miR-21. He is interested in exploring the idea that miR-21 could be a therapeutic target on the order of Bcr–Abl in chronic myeloid leukemia.

“In the case of the ABL kinase, one gene appears to play a more important role than all the other genes that are mutated,” said Slack. “They’ve become so dependent on having that gene be active that if you just take that away, the cells die.” He hopes that miR-21 will present a therapeutic target similar to the ABL kinase.

**miR-21 and Trastuzumab**

Other researchers are also intrigued by miR-21 and Trastuzumab. "They've become so dependent on having that gene be active that if you just take that away, the cells die.” He hopes that miR-21 will present a therapeutic target similar to the ABL kinase.

Other researchers are also intrigued by miR-21's potential—and so far hypothetical—clinical applications. At the M. D. Anderson Cancer Center in Houston, Dihua Yu, M.D., Ph.D., and her graduate student Sumaiyah Rehman found that high miR-21 levels in patient breast tumor samples correlated with disease progression and with poor patient response to trastuzumab (Herceptin), which targets Her2.

Yu said that recent studies showing that miR-21 can reduce the level of the tumor suppressor phosphatase and tensin protein (PTEN) in hepatocellular carcinoma cells led her group to look at PTEN levels in breast tumors.

Using three Her2-positive breast cancer cell lines, the researchers introduced miR-21 by using a commercially available transfection vector. Cells with higher levels of miR-21 had reduced PTEN expression and were resistant to treatment with trastuzumab. Conversely, cells in which miR-21 was knocked down had increased PTEN expression and were more sensitive to the drug than were control-group cells. Yu said these results suggest that miR-21 levels may help determine which patients are more likely to respond to trastuzumab. She and Rehman reported their results at the American Association for Cancer Research meeting in April.

Meanwhile, Yu said, her lab continues to work out miR-21’s other targets in a xenograft mouse model of breast cancer. The reduced PTEN expression in response to miR-21 was not dramatic, she says, and it is probably not the only player involved in the response. In fact, miRNAs have multiple targets, and unlike the similar-sized double-stranded small-interfering RNAs (siRNAs), they show weaker binding to more mRNA targets.

Yu said that she expects the response in breast cancer cells is multifaceted. She speculated that it may end up being one of several markers used to create personalized patient profiles to guide treatment.

Indeed, much of the experimental work with miRNA regulation suggests that these molecules tend to inhibit several targets weakly in a network of effects. This, said miRNA researcher Joshua Mendell, M.D., Ph.D., makes them excellent prospects as therapeutic agents because their multiple effects make development of resistance less likely than with a single-target agent.

**miR-26a and Liver Cancer**

Mendell, of Johns Hopkins University in Baltimore, is focusing on miRNA that becomes suppressed in cancerous states. One of these, miR-26a, is found at high levels in most human tissues and organs, but its expression is repressed in liver cancer, a tumor with few effective therapeutic options and a 5-year survival rate of less than 14%. miR-26a targets cell cycle genes and genes involved in programmed cell death.

Mendell and colleagues developed an adenoviral-based delivery system to administer miR-26a systemically in a transgenic mouse liver cancer model. When the adenoviral virus enters cells, its genetic cargo forms a circular, self-contained epimere that operates independently of the animal genome; miRNA-26a is then transcribed from the epimere. The researchers injected the viral vector into 10 experimental and eight control mice in which tumors were induced by the oncoprotein MYC. After 3 weeks they assessed the tumor burden in each animal. In eight of 10 treated animals, tumors regressed dramatically. Further inspection demonstrated that the two failed experimental animals had inefficient transfection rates rather than resistance to the treatment. All control animals developed aggressive liver tumors.

Just as important, Mendell said, the animals showed no signs of the acute liver toxicity that has plagued other attempts to target the miRNA pathway by using synthetic siRNA. Mendell attributes the lack of toxicity to improvements to the vector and to the fact that miR-26a is normally found at high levels in mammalian tissues. In contrast, the immune system can mistake
synthetic double-stranded siRNA for viral genetic material and induce an immune response.

“We are really restoring a natural tumor suppression mechanism,” he said. “That’s an important contrast between siRNAs and miRNAs. In the case of an siRNA, we are designing a synthetic molecule that acts as a drug, really, that is trying to silence an oncogenic gene product. But a microRNA is really trying to restore a natural tumor-suppressive mechanism.”

Results of these experiments, published in *Cell* in June 2009, encouraged the researchers to move toward clinical trials.

“We are very optimistic that we will be able to bring this forward for a phase I safety trial,” Mendell said. “We have all the relevant clinicians and scientists in place. We have the resources to produce the clinical-grade vector that meets FDA requirements.” The group, led by investigators at Children’s Hospital in Columbus, Ohio, has submitted grant applications to do a phase I trial.

Mendell acknowledges that restoring a tumor-suppressive mechanism does not address the underlying cause of the cancer—but, he said, it may not matter. He pointed out that in the MYC mouse model, “when we got the microRNA into the tumor cells, it killed the cells. It actually eliminated the tumor cells.” Whether this specific therapy with miR-26a will work only in tumors that are started by activation of MYC is unknown, Mendell added. “I think that’s an important question for future work.”

Dr. Slack serves as a scientific advisor to Mirna Therapeutics, which is developing and commercializing miRNA therapeutics.

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