Small RNAs Are Raising Big Expectations

By Karyn Hede

Five years ago there were little more than 200 known microRNAs (miRNAs)—small regulatory nucleic acids that bind to complementary sites on mRNAs and prevent their translation into protein. Today, that number has swollen to more than 700 and counting. The explosion in knowledge about these small, noncoding RNAs, which appear to be evolutionarily ancient but were discovered less than two decades ago, has already led to the marketing of new cancer diagnostic tools and, researchers say, may eventually lead to treatments.

Take the case of “cancers of unknown primary” (CUPs)—cancers that clearly originated in another, unknown site. CUPs account for about 3% of diagnoses according to Robert Hromas, M.D., chief of oncology services at the University of New Mexico Cancer Research and Treatment Center in Albuquerque.

Using a new miRNA-based test, physicians can now identify the site that the CUP tumor came from, Hromas said, allowing them to choose an appropriate chemotherapy regimen. That test is one of three new miRNA-based cancer diagnostic tests now marketed by Rosetta Genomics in Rehovet, Israel, a diagnostics company with a U.S.-based testing facility in Philadelphia. The tests are designed to differentiate cancers on the basis of the levels of a subset of miRNA associated with a particular form of cancer.

Clinical testing, which was just completed for at least one of the tests, showed that miRNA profiling distinguished squamous cell lung carcinomas from other non-small-cell lung cancers with 96% sensitivity and 90% specificity in 122 adenocarcinoma and squamous cell carcinoma samples.

The Rosetta tests may be the first in a wave of miRNA-based assays. At the American Association for Cancer Research (AACR) meeting in April, more than 200 posters and presentations contained data linking miRNA to just about every known form of cancer. Researchers say they hold promise now, not only as diagnostics but also as prognostic biomarkers.

Prognostic Potential

For example, in one recent study, doctors at Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y., found that miRNA patterns were associated with poor outcomes in patients with head and neck cancer. Tumor samples and adjacent healthy tissue from 104 patients with head and neck cancer were tested for miRNA levels. After a 5-year follow-up, patients with the lowest levels of the miRNAs called miR-205 and let-7d were four times more likely to have early metastasis or locoregional recurrence of their cancer than patients with higher levels, according to the findings published recently in the American Journal of Pathology.

Richard Smith, M.D., vice chair of head and neck surgery at Einstein, said that the ability to identify more aggressive tumors would allow clinicians to more appropriately treat tumors. The researchers are now trying to confirm their result in a new patient group and looking for other markers that would make the test a reliable prognostic tool.

The excitement over miRNAs as a biomarker is reminiscent of previous protein and mRNA biomarkers that showed promise as prognostic tools but didn’t pan out in the clinic. However, miRNA has some important differences from other types of cancer biomarkers, said Thomas Schmittgen, Ph.D., of Ohio State University’s School of Pharmacy in Columbus.

“There appear to be unique cell and tissue type expression patterns of miRNA, which is what you’d want for a diagnostic,” he said. “They appear to be very stable in biological fluids, including serum. And there are very sensitive assays like PCR that can be used to detect them. But the big advantage is that you are profiling a much smaller number of genes than you are with whole-genome profiling.”

For reasons that are still not well understood, a small number of miRNAs has consistently given a more accurate diagnosis of cancer type than much larger samples of mRNA transcripts, said Schmittgen. He is working on an miRNA test to diagnose pancreatic cancer from a small blood sample and to standardize miRNA testing to make it reproducible in any diagnostic laboratory. His initial studies, published in 2006, showed that a panel of miRNA could correctly differentiate among pancreatic tumor, adjacent normal tissue, and normal tissue from people without pancreatic cancer with near-100% accuracy. Now Schmittgen is working to replace invasive fine-needle aspiration of a suspect pancreatic mass with a much less invasive blood sample.

“There is accumulated evidence now that miRNAs have potential to be significant prognostic indicators,” said Curtis Harris, M.D., chief of the laboratory of human carcinogenesis at the National Cancer Institute. The next step is to validate these results in prospective studies.

The key to effective miRNA diagnostic tests will be to combine them with other...
biomarkers to reduce misclassification errors, Harris said. He and his colleagues presented data at AACR demonstrating that combining miRNA data and levels of inflammatory cytokines is predictive of colon cancer. The findings follow on the group’s January 2008 publication in the *Journal of the American Medical Association* showing that a high level of miR-21 is associated with poor outcome in colon cancer patients. With combined biomarkers, each will misclassify some patients, he said. But the combination should reduce misclassification errors.

**Mining the “miRNAome”**

Some investigators have gone beyond associating miRNA levels with cancer to examining the repercussions of alterations in the machinery that processes miRNA. Xifeng Wu, M.D., Ph.D., at the University of Texas M. D. Anderson Cancer Center in Houston, presented data at AACR that link genetic variations in miRNA processing genes and in miRNA binding sites to ovarian cancer risk and patient outcome. The concept, said Wu, is that dysfunctional miRNA processing alters gene regulation to promote tumorogenesis.

In their most recent study, Wu and colleagues examined single-nucleotide polymorphisms, or SNPs, in the miRNA-processing genes of 417 ovarian cancer patients and an equal number of healthy control subjects. They found 21 SNPs that were statistically significantly associated with overall survival. The median survival of patients with six or fewer unfavorable genetic alterations was 151 months, versus 24 months for those with 10 or more. One variation stood out as being particularly important. Patients with alterations in the miRNA binding site for the gene encoding platelet-derived growth factor C (PDGFC), a vascular endothelial growth factor–like protein, were less likely to respond to platinum-based chemotherapy, the most common treatment for ovarian cancer.

“Eventually we are going to use this information and incorporate additional information to build a personalized risk prediction model,” said Wu. “So when a patient is diagnosed with ovarian cancer, the doctor would ask for a small blood sample and very quickly, within a couple of hours or at most a couple of days, [the results] would inform the physician whether that patient will respond to the platinum-based chemotherapy.” Also, Wu hopes that the model will help identify individuals at higher risk of developing ovarian cancer so that they can be screened more frequently. She said that M. D. Anderson is working on a patent for the model but has no commercial partners yet.

**From Diagnosis to Therapy**

The ultimate goal of many investigators is to take miRNA from a diagnostic tool to a therapeutic agent. Doing so will require more knowledge of the specific genes that are targets of miRNA regulation, but already a few studies hint at therapeutic targets.

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Aurora Esquela-Kerscher, Ph.D.

Dipanjan Chowdhury, Ph.D., and his colleagues at the Dana–Farber Cancer Institute in Boston reported in the May issue of *Nature Structural and Molecular Biology* that an miRNA, called miR-24, dampens the ability of mature white blood cells to repair DNA damage by downregulating expression of the DNA repair protein H2AX. Chowdhury believes that miR-24’s role is to turn off proteins that control the cell cycle in rapidly dividing cells once those cells reach a mature, quiescent stage.

To test the effect of miR-24 on DNA repair, the researchers introduced miR-24 to K562 leukemia cells and showed that they became hypersensitive to both bleomycin and cisplatin chemotherapy agents. The researchers reversed the effect by introducing a form of H2AX that miR-24 does not recognize. Chowdhury, who collaborated with scientists at Rosetta Genomics on the research, said that the findings may lead to new tests for determining a tumor’s aggressiveness and the likelihood of response to radiation and chemotherapies.

“Before this report there are no published reports of miRNA impacting DNA repair,” said Chowdhury. The next step, he said, is to determine which specific miRNAs influence sensitivity to radiation therapy. Once that information is available, he added, “let’s go and see if we can modulate these. I do believe they would be therapeutic targets.”

Other experts express caution. What’s needed now, said Aurora Esquela-Kerscher, Ph.D., assistant professor of microbiology and molecular cell biology at Eastern Virginia Medical School in Norfolk, is a better understanding of their functional properties. “Very few of the [700 miRNAs] discovered to date have been biologically characterized. People are very good at finding them, and validating them, and seeing how they are expressed up or down in various diseases, but what’s really lacking in the field right now is their functional properties.”

Of the 50 or so miRNAs that have been carefully characterized, all appear to be involved in cell differentiation and cell cycle control, she said. So knowing what their targets are before attempting to manipulate them therapeutically will be important.

Indeed, despite the proliferation of studies, most experts would agree that the field is still young. Until 1993, no one knew this fundamental regulatory network existed, said NCI’s Harris. And the initial data suggesting that miRNA might be important in cancer started showing up in the literature only around 2004. “In a very short time there’s been a lot of progress,” he said.