



Take a tour of our
"Life Sciences a

Feature Articles

February 15, 2017 (Vol. 37, No. 4)

Reading miRNAs for Clinical Meaning

Single miRNAs Don't Say Much, but They May Become Eloquent Biomarkers if We Take Their Testimony in Context

Richard A. Stein, M.D., Ph.D.

Soon after it was established that less than 2% of the human genome encodes for proteins, it was supposed that the vast majority of genetic material was mere junk. Subsequently, after advances to genomics and transcriptomics technology, scientists realized that the remaining 98% of the genome contains sequences that perform key regulatory functions.

Some of these sequences give rise to microRNAs (miRNAs), small noncoding RNA molecules that have emerged as one of the most complex, multilayered, and intriguing constituents of gene-regulatory networks.

Biomarkers and Therapeutics

"We are studying microRNAs on two fronts," says Jingfang Ju, Ph.D., professor of pathology at the State University of New York at Stony Brook. "We are evaluating microRNAs as cancer biomarkers and as therapeutic molecules."

In a recent study, Dr. Ju and colleagues focused on Dickkopf 4 (DKK4), an inhibitory molecule of the WNT/ β -catenin signaling pathway. DKK4, the investigators determined, has a role in the development and progression of pancreatic cancer. Using immunohistochemistry and qRT-PCR, Dr. Ju and colleagues learned that while DKK4 is normally at undetectable levels in normal pancreatic cells, it is highly overexpressed in pancreatic cancer cells.

A transcriptome sequencing method to examine the molecular mechanisms that underlie DKK4 overexpression identified several differentially expressed genes in a pancreatic cell line, and a gene ontology analysis revealed that most upregulated genes were involved in cellular immune responses, inflammation, cellular proliferation, cell motility, and tumor-associated signal transduction. Pathway analysis identified the MAPK pathway as the main signaling transduction pathway in DKK4-overexpressing pancreatic cancer cells.

The investigators reported that miR-15a and miR-506 play key roles in pancreatic cancer progression and resistance.

"The goal in biomarker development is to use microRNA expression-based biomarkers to better manage the clinical treatment of cancer," declares Dr. Ju. Historically, mRNA expression, DNA mutations, and proteins have been used as the most common biomarkers.

"About 10 years ago, we discovered that a unique feature of microRNAs is their superior stability in archival formalin-fixed paraffin-embedded (FFPE) tissues," recalls Dr. Ju. Hospitals have large repositories of paraffin blocks of patient samples that are kept for decades, and these provide a valuable resource for clinical information in terms of diagnosis, treatment, clinical course, and therapeutic outcome.

To capitalize on the ability of miRNAs from paraffin-embedded tissues to provide actionable information, Dr. Ju and colleagues started using FFPE samples to conduct large, archival, retrospective studies on colorectal cancer patient samples, and identified several miRNAs that can predict response to chemotherapy and survival. "We have transformed those biopsy samples into treasures thanks to the stability of miRNAs," says Dr. Ju.

In a study that examined about 200 patient samples, Dr. Ju and colleagues validated the prognostic value of a panel of miRNAs and also incorporated tumor location information (left vs. right side) into the miRNA expression data. "This analysis," states Dr. Ju, "revealed that the combination of microRNA expression and tumor location provide a better prognostic indicator than microRNA expression alone."

Dr. Ju and colleagues cross-validated these findings by using the RNA-Seq results from The Cancer Genome Atlas (TCGA) colorectal cancer database. While much work in Dr. Ju's lab has focused on colorectal cancer, additional efforts are exploring miRNAs in other gastrointestinal malignancies, including gastric cancer and pancreatic cancer. "We would like to push this to help oncologists better manage treatment," remarks Dr. Ju.

In an effort to generate therapeutic miRNA molecules, Dr. Ju and colleagues introduced several novel modifications into miRNA. One of the modifications took advantage of RNA's incorporation of uracil instead of thymine residues.

"Because 5-fluorouracil (5-FU) is the major chemotherapy agent used in colorectal cancer, we wanted to integrate the therapeutic power of 5-FU with the tumor suppressive nature of miRNA," explains Dr. Ju. To achieve this, Dr. Ju and colleagues generated a miRNA therapeutic molecule in which the uracil was replaced with 5-FU. The newly generated miRNA retained its target specificity with enhanced stability.

"We integrated the two drugs and found that the modified microRNA was much more potent than the nonmodified one," says Dr. Ju. "Also, the modified microRNA was over 100-fold more potent than 5-FU alone."

The work accomplished by Dr. Ju's team has attracted the support of the Long Island Bioscience Hub, an NIH-designated Research, Evaluation, and Commercialization Hub (REACH). "With this support," comments Dr. Ju, "we are hoping to accelerate the translation of drug discovery to improve patient care and enhance health."