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Abundance of New MicroRNAs Not a Mirage

Using a new analytical technique called miRAGE, researchers have discovered 133 new microRNAs in the largest analysis of human microRNAs to date. The new bounty increases the number of experimentally verified microRNAs by almost 50 percent and provides researchers with new tools for understanding how genes are regulated.

MicroRNAs are tiny molecules that regulate the expression of genes. They do not produce proteins, but they control the RNAs that do and thus are key players in many physiological and developmental processes. The researchers said their findings suggest that many more microRNAs (miRNAs) remain to be discovered in other tissues throughout the body. Such discoveries would likely spur fresh insights into how miRNAs control gene expression and possibly illuminate differences in miRNA regulation between normal and cancerous cells.

The researchers will publish their findings in the March 7, 2006, issue of the *Proceedings of the National Academy of Sciences (PNAS)*. The article was published in the *PNAS* online early edition on February 27, 2006. Victor Velculescu of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins and Howard Hughes Medical Institute investigator Bert Vogelstein at Johns Hopkins were senior authors of the paper. Jordan Cummins, who works with both Velculescu and Vogelstein, was the first author of the paper.

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- Jordan Cummins

Discovered a little more than a decade ago, miRNAs have rapidly become the focus of considerable scientific scrutiny. However, the regulatory roles of the tiny RNAs have remained mysterious because researchers have only managed to pin down the function of a handful of the more than 200 known miRNAs. Unlike the larger messenger RNA (mRNA) molecules that carry

genetic information from genes to the protein-production machinery, miRNAs regulate gene activity by interfering with the genetic message contained in mRNA molecules.

“MiRNAs are a new class of genes implicated in organismal development and a host of human diseases, including cancer,” said Cummins. “This new approach has enabled us to perform the largest analysis of human miRNAs to date, both quantifying known miRNAs and discovering novel miRNAs, and increasing the number of known miRNAs by almost fifty percent. The study shows that previous predictions for an upper limit to the total number of human miRNAs were too low.”

According to Velculescu, many scientists believed that the current techniques researchers are using to discover microRNAs had uncovered just about all the existing miRNAs in the cell. Those techniques led to the discovery of some 321 genes for human miRNAs. However, he said, both of the major search techniques had drawbacks that could limit discovery of new miRNAs. The technique of cloning individual genes to determine whether they coded for miRNAs was laborious and time-consuming. And the bioinformatics approach of using computers to scan the human genome to predict sequences that might code for miRNAs required experimental verification of those predictions, he said.

Cummins, Velculescu, Vogelstein and their colleagues decided to develop and apply a new mass analysis technique to search for miRNAs in colorectal cells. Colorectal cancer has been a central focus of research for Velculescu and Vogelstein.

They called their technique “miRNA serial analysis of gene expression,” or miRAGE. Basically, it involves isolating thousands of tiny RNA molecules from cells. These RNA molecules are reverse-transcribed into DNA molecules, and these are then linked together into much larger chains. Researchers are then able to sequence the longer DNA molecules in a way that is much more efficient than if they had started with shorter DNA sequences derived from individual RNA molecules.

The researchers next used computer algorithms to analyze the DNA sequences to recognize those that had characteristics of genes that code for miRNAs. They also applied other criteria to validate that the genes coded for miRNAs. For example, they determined whether candidate human genes resembled known miRNA genes in other species; or whether the miRNAs they produced had molecular characteristics of functioning miRNAs. Such characteristics include the ability of the candidate RNA molecule to form a “hairpin” that is recognized by the enzyme Dicer, a molecule that snips precursor microRNA molecules into their mature, functional form.

“Using these criteria, we found a total of a hundred and sixty eight putative novel microRNAs,” said Velculescu. “However, during the course of the

study, thirty five of these were independently identified by other approaches as being microRNAs, which left us with 133 new microRNAs. The ones confirmed by these other approaches provided very nice confirmation that we were, indeed, identifying microRNAs that hadn't been in the database originally but were legitimate microRNAs," he said. In addition, noted Velculescu, the miRAGE technique identified 200 known miRNAs.

In a separate approach to validating candidate miRNAs, the researchers compared the generation of mature miRNAs in normal cells and cells depleted of the Dicer enzyme. The researchers found significant reduction in levels of mature miRNAs for many of the candidates in the Dicer-depleted cells.

"This not only gives us another layer of validation, showing that these are legitimate microRNAs, but it also substantiates the idea that Dicer really is critical for the biogenesis of at least some fraction of known and novel microRNAs," said Velculescu.

In addition to the 133 new miRNAs, the researchers also found 112 previously unrecognized miRNA "star forms." These are miRNA strands that are the complementary strands of the functional single-stranded miRNAs. They are initially part of the double-stranded miRNA molecule before processing by Dicer, and are generally degraded by the cell. Until now, few star forms had been found, and the discovery of so many new star forms is important evidence for their presence in human cells, said Velculescu.

According to Cummins, the discovery of a multitude of new miRNAs in colorectal tissues represents only the beginning of a broader hunt throughout the body. "Since microRNA expression tends to be tissue-specific, a thorough analysis of diverse human tissues using a high throughput method such as miRAGE will help bring us closer to the full compendium of human microRNAs," he said.