It's a trap! New laboratory technique captures microRNA targets

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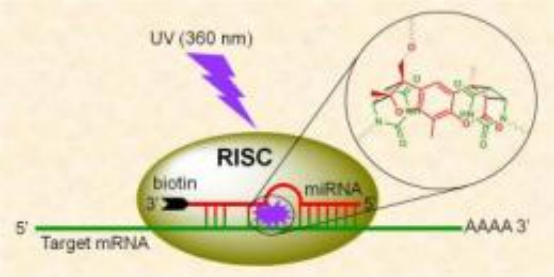

MicroRNAs suppress gene expression by binding target mRNA and joining an RNA-induced silencing complex. A simple new technique called miR-TRAP captures the complex, allowing researchers to more easily identify an miRNA's target. Credit: Sanford-Burnham Medical Research Institute

Human cells are thought to produce thousands of different microRNAs (miRNAs)—small pieces of genetic material that help determine which genes are turned on or off at a given time. miRNAs are an important part of normal cellular function, but they can also contribute to human disease—some are elevated in certain tumors, for example, where they promote cell survival. But to better understand how miRNAs influence health and disease, researchers first need to know which miRNAs are acting upon which genes—a big challenge considering their sheer number and the fact that each single miRNA can regulate hundreds of target genes. Enter miR-TRAP, a new easy-to-use method to directly identify miRNA targets in cells. This technique, developed by Tariq Rana, Ph.D., professor and program director at Sanford-Burnham

Medical Research Institute (Sanford-Burnham), and his team, was first revealed in a paper published May 8 by the journal *Angewandte Chemie International Edition*.

"This method could be widely used to discover miRNA targets in any number of disease models, under physiological conditions," Rana said. "miR-TRAP will help bridge a gap in the RNA field, allowing researchers to better understand diseases like cancer and target their genetic underpinnings to develop new diagnostics and therapeutics. This will become especially important as new high-throughput RNA sequencing technologies increase the numbers of known miRNAs and their targets."

How miR-TRAP works

miRNAs block gene expression not by attaching directly to the DNA itself, but by binding to messenger RNA (mRNA), the type that normally carries a DNA recipe out of the nucleus and into the cytoplasm, where the sequence is translated into protein. Next, these RNAs are bound by a group of proteins called the RNA-induced silencing complex, or RISC. This blocks production of the protein encoded by that mRNA, an action that can have far-reaching consequences in the cell.

miR-TRAP is performed in three basic steps. Scientists 1) produce highly photoreactive probes by conjugating psoralen, a plant molecule that can be activated by light, to an miRNA of interest, 2) perform a long-wave UV photocrosslinking reaction, and 3) pull down RNA and analyze it by RT-qPCR. In other words, researchers zap cells with UV light, freezing the miRNA/mRNA duo in place. Then, after extracting the RNA from the cells, they can take a closer look at the sequence of the bound mRNA, revealing the miRNA's target gene.

Advantages of miR-TRAP

miR-TRAP is easier and more accurate than current methods of identifying miRNA targets for three main reasons. First, miR-TRAP can directly identify miRNA targets in live cells, under normal or disease conditions. Second, this technique can spot mRNA targets that are not only reduced by miRNAs, but also those whose translation into protein is repressed—targets that aren't normally picked up by other techniques, such as qPCR or microarray analysis. Third, miR-TRAP doesn't rely on antibodies, which can lead to nonspecific background signals and complicate data interpretation.

Putting miR-TRAP to the test, Rana and his team, including postdoctoral researcher Huricha Baigude, Ph.D., analyzed 13 predicted targets of two important microRNAs. The technique not only confirmed their known gene targets, but also revealed two novel targets.

"We're now applying these methods to identify miRNA targets in a number of disease models," Rana said. "And it's our hope that miR-TRAP will soon become common practice in many labs around the world."

Provided by Sanford-Burnham Medical Research Institute