

FEBRUARY 01, 2008

New Genetic Barcoding Technique Identifies Dozens of Targets for Cancer Drugs

Howard Hughes Medical Institute (HHMI) investigators have invented a quick and relatively inexpensive method for identifying genes that are indispensable for the growth and survival of colon and breast cancer cells.

The approach employs a massively parallel cellular system that simultaneously screens thousands of genes. Researchers can use information from the genetic screen to assess the relative impact of each gene on the growth and survival of tumor cells.

In two papers published in the February 1, 2008, issue of the journal *Science*, Gregory J. Hannon and Stephen J. Elledge describe their new screening system and identify dozens of potential new gene targets for fighting colon and breast cancer. The researchers hope that their approach will help researchers develop new drugs that selectively kill cancer cells.

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- Stephen J. Elledge

"We're finding all kinds of potential new targets in our gene lists that are specific for killing cancer cells but don't seem to affect normal cells," says Elledge, an HHMI investigator and the Gregor Mendel Professor of Genetics at Harvard Medical School.

Even better, says Hannon, who is an HHMI investigator at Cold Spring Harbor Laboratory, is that the technique is simple enough that "any investigator can do it without millions of dollars of robotics and other high-tech equipment."

The method exploits a powerful cellular mechanism called RNA interference. Discovered just a decade ago, RNA interference likely evolved to help cells fight viruses. The cellular machinery involved in RNA interference first identifies short segments of suspicious-looking RNA, and then destroys all identical copies of that RNA. The result: None of the protein that the RNA encodes for gets made.

While RNA interference prevents viruses from replicating inside cells, scientists discovered that they could exploit the process to squelch individual gene products. To do so, they introduce a short segment of RNA that looks like one of the cell's normal genes. The RNA interference machinery grinds into action and shuts down production of the protein made from that gene.

Six years ago, Elledge and Hannon began making a library of RNAs, called short hairpin RNAs, which trigger the RNA interference mechanism. They've now made short hairpin RNAs that can squelch every gene in the human and mouse genomes.

For their new experiments, the pair first identified about 3,000 genes important in cell signaling, growth, and other essential processes. Next, they inserted a gene sequence coding for short hairpin RNAs targeting these genes into retroviruses. Then they infected dishes of normal and tumor cells with the retroviruses, which added instructions to each cell's genome telling it to produce a short hairpin RNA. These short RNAs then triggered the RNA interference mechanism. In effect, each virus halts production of a single protein in a single cell.

In the past, researchers deployed this method to study the effects of turning off one particular gene. But to study the effects of thousands of genes, researchers had to run thousands of separate experiments with thousands of plates of cells.

Instead, Hannon and Elledge developed a "barcoding" method to track a diverse pool of short hairpin RNAs in parallel. In the barcoding method, every short hairpin RNA that is made carries a unique genetic tag. This tag lets the researchers track the effect of thousands of the RNAs in a single pool of cells in a single lab dish.

"We get a mixture of cells where each individual cell has one of these genes knocked down," says Hannon.

If RNA interference knocks down a gene important for cell growth and survival, the cell fails to thrive or dies. At the end of the experiment, the researchers recover only small amounts of the short hairpin RNA associated with that gene. The researchers then know that the gene is a potential Achilles heel for the cell.

In the research reported in the *Science* papers, the scientists ran many such experiments on normal, breast, and colon cancer cells. The team found dozens of genes that, when eliminated, hinder cancer cells but don't seem to harm normal cells.

“We're examining as many genes as we can, and eventually every gene in the genome, to figure out which ones are deleterious to tumor cells. And when you screen in this unbiased way, you start finding things you couldn't have predicted,” says Elledge.

He adds that this kind of functional screen -- to see which genes will kill cancer -- is complementary to the \$1.5 billion government effort to sequence the genomes of various types of cancer cells.

“If you take all the sequences that will come out of the expensive cancer genome sequencing effort, you're not going to know which ones are important until you do functional analysis,” says Elledge. “We're already doing that functional analysis.”

Hannon and Elledge are making their library of short hairpin RNAs available to researchers through a collaboration with Open Biosystems, a company based in Huntsville, AL.

“We have a dual goal,” says Hannon. “We want to advance our own science, but we want others to use these tools too. The utility of the method is limited only by the creativity of the scientist using it.”