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New Strategy Rapidly Identifies Cancer Targets

In a step toward personalized medicine, scientists have used a new technique to identify genetic mutations that can trigger cancerous growth. By analyzing the proteins - instead of the genes - inside acute myeloid leukemia (AML) cells, the researchers have dramatically reduced the time it takes to zero in on molecular abnormalities that might be vulnerable to specific drug treatments.

Howard Hughes Medical Institute investigator Brian J. Druker and colleagues collaborated on the research with scientists at Cell Signaling Technology, who developed the technique for protein analysis. Scientists in the lab of D. Gary Gilliland, an HHMI investigator at Brigham and Women's Hospital, as well as researchers at the Portland VA Medical Center, the University of Chicago, and Yale University also participated in the research, which was published in the July 17, 2006, issue of the journal *Cancer Cell*.

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— **Brian J. Druker**

This approach gives us a way to figure out what's driving the growth of a cancer in an individual patient and ultimately match that patient with the right drug, said Druker, who is based at the Oregon Health & Science University Cancer Institute in Portland.

Traditionally, cancer-gene hunters have scanned the genome looking for mutations that trigger out-of-control cell growth. Druker tried this approach, but found it wanting. We were doing some high-throughput DNA sequencing, and we weren't really finding much, he said.

Instead, the team added tools from the burgeoning field of proteomics, the study of proteins. We decided this more functional assay would get us to the disease-causing genes more rapidly, said Druker, who has been studying a group of cell-signaling proteins called tyrosine kinases for 20 years.

Tyrosine kinases play a key role in many cancers. In healthy cells, they help form a chain of signals that prompt normal cell growth and division. Sometimes, though, a tyrosine kinase gets stuck in an on position, driving out-of-control cell division and, ultimately, cancer. This potentially devastating kinase activation carries a calling card in the form of a molecule called a phosphate.

The phosphates signal activated tyrosine kinases, said Druker. So we decided to use the phosphates as markers.

Using Cell Signaling Technology's proteomics method, the team was able to find these markers in myeloid leukemia cells. The first step was to chemically digest the cells into a mixture of protein snippets called peptides. Next, they extracted all of the peptides carrying extra phosphates and sent them through a mass spectrometer, which precisely measured the weight of each peptide. Sophisticated software then sifted through a massive protein database at the National Library of Medicine, identifying each of the team's peptides as a segment of a specific protein. The analysis showed that many of the peptides came from tyrosine kinases. Scanning this list, Druker and his collaborators picked out five as likely suspects.

Druker's team then introduced into their leukemia cells five segments of RNA that each shut down one of the candidate kinases. Silencing four of the kinases with RNA did nothing - the cells still grew out of control. But with the fifth, the cells no longer became cancerous.

That left one gene to sequence. We found that the gene, called *JAK3*, had a mutation that drives the growth of leukemia cells in mice, said Druker. Analysis of additional patient samples later identified two more mutations in the *JAK3* gene.

Thomas Mercher, a postdoctoral fellow in Gilliland's lab, then tested the mutation in a mouse model. It was important to show that the *JAK3* mutation, when introduced in mice, would lead to a leukemia-like illness. It did, confirming that the *JAK3* mutations play a central role in leukemia, said Gilliland.

While Druker said that only a small proportion of AML patients likely carry *JAK3* mutations, he says the technique will help find other cancer-causing mutations. The process is highly technical, but it cuts the time needed to find a faulty gene by months. If you try to sift through DNA, it takes almost a year, said Druker. This technique takes a couple of months and further automation would make it even quicker.

So quick, in fact, that Druker envisions the technique as a method to help choose which drug a patient's cancer will respond to - a critical step in achieving personalized medicine. Four cancer drugs that inhibit tyrosine kinases are already on the market, including Gleevec, which Druker pioneered for treatment of chronic myelogenous leukemia. Since its approval in 2001, Gleevec has made CML a much more manageable disease.

A new drug to inhibit JAK3 is already in development elsewhere, and, eventually, Druker sees a market filled with a dozen or more tyrosine kinase inhibitors. Matching the patient to the right drug will then simply be a matter of running their cancer cells through a future version of their new technique. Druker is already testing the idea. Now we know to add *JAK3* to the mutations we screen for in our leukemia patients and should look to see if *JAK3* mutations are present in other cancers, he said.