MedicalXpress

New Stanford diagnostic test for rare leukemia appears to give faster results, study finds

October 6 2008

A new twist on a well-known cell sorting technique may allow physicians to diagnose rare leukemias in hours instead of weeks, according to a study by researchers at the Stanford University School of Medicine and UC-San Francisco. The clinical promise of the Stanford-developed approach, which eavesdrops on individual cells to decipher potentially dangerous molecular conversations, is likely to extend to many other disorders in which cell-signaling pathways are disrupted.

"We've eliminated a big bottleneck," said postdoctoral scholar Nikesh Kotecha, PhD, of the work. Kotecha, the lead author of the study, conducted the work as a graduate student in the laboratory of immunologist Garry Nolan, PhD, a co-senior author of the paper. "Now we can use this signaling assay to confirm a diagnosis much more quickly."

The study will be featured on the cover of the Oct. 7 issue of *Cancer Cell*. In addition to Nolan, who is an associate professor of microbiology and immunology as well as a member of Stanford's Cancer Center, the other senior co-author is Mignon Loh, MD, an associate professor of clinical pediatrics at UCSF.

"We couldn't have done this research without involving immunology, signaling biology, medicine, statistics and informatics," said Kotecha, who completed his PhD in biomedical informatics. "It's a true example

of the strengths of translational research, bringing laboratory bench work and informatics to address a clinical problem."

The multidisciplinary technique builds on an experimental technique called flow cytometry, in which fluorescently labeled antibodies are used to classify and sort cells based on proteins displayed on their outer surface. The new approach, developed in Nolan's lab in 2004, creates small holes in the cell membrane prior to sorting. These holes allow other antibodies to enter the cell and bind to signaling molecules involved in the cell's internal monologue — in this case, a protein called STAT5. Kotecha and his collaborators used an antibody that binds only to the activated, or phosphorylated, version of the protein to determine the signaling status of the pathway in individual cells exposed to a variety of conditions.

The effect is somewhat like moving through an airport security line that screens travelers not just for weapons concealed outside their bodies, but also for their emotional states: "Happy to be headed home" in one line, "afraid of flying" in another and just plain "cranky" in another. Combine the two measurements — the availability of a weapon and the mood of the person carrying it — and you have a more reliable assessment of risk than with either one alone.

The researchers tested the technique's clinical value by applying it to the diagnosis of a difficult diagnostic problem: juvenile myelomonocytic leukemia, or JMML. Children with the relatively rare disorder typically have fevers, grow poorly, suffer from infections and generally look like they could have any one of a number of different diseases. A prompt diagnosis of JMML is particularly important because, unlike other leukemias, the only cure is a bone marrow transplant.

One of the few reliable indicators of JMML cells is their tendency to proliferate in response to very low levels of a growth-stimulating factor

called GM-CSF; normal cells respond only at higher levels. But it can take two to three weeks to grow enough cells in the laboratory to get a definitive answer to this test.

Kotecha knew that GM-CSF activates a particular cellular signaling cascade called the JAK-STAT pathway. Although that pathway had not previously been directly implicated in JMML, Kotecha used an antibody that binds only to activated STAT5 to determine whether the cells of 12 patients with JMML displayed abnormally high levels of the protein in response to low doses of GM-CSF. Eleven of the 12 did so — confirming the involvement of the STAT pathway in the disorder.

"I was surprised how much more we can learn about the inner nature of these cells by 'interrogating' them with different conditions," said Nolan, who is also a member of the Donald E. and Delia B. Baxter Laboratory in Genetic Pharmacology at Stanford. "Time and again we are finding this to be a powerful amplifier of the fate of a diseased cell and a good way to understand why it responds to certain treatments and not others."

In contrast to the JMML samples, seven out of eight normal bone marrow samples, as well as eight out of eight samples from patients with similar, but not identical, disorders, maintained normal levels of activated protein after the low dose GM-CSF treatment — suggesting that the technique may be a sorely needed diagnostic aid for JMML.

The new technique also offers a way to monitor disease progress. With further refinement, the researchers hope that the technique can be used to screen the effectiveness of potential drugs for treatment of JMML and other disorders.

"Identifying populations of cells by their response to specific stimuli will facilitate our ability to assess the efficacy of specific agents in relevant subsets with increased precision," said Loh. "In an era of using

increasingly sophisticated targeted agents, we hope that these studies will allow investigators to more fully appreciate the specificities of their therapies."

Source: Stanford University Medical Center