Advanced instrument used to read cells' minds

May 5 2011

The CyTOF instrument used to carry out mass cytometry. The apparatus can measure the physical traits of as many as 1,000 single biological cells per second. Credit: Image credit: Sergey Vorobiev, DVS Sciences Inc.

Researchers at the Stanford University School of Medicine have taken a machine already in use for the measurement of impurities in semiconductors and used it to analyze immune cells in far more detail than has been possible before. The new technology lets scientists take simultaneous measurements of dozens of features located on and in cells, whereas the existing technology typically begins to encounter technical limitations at about a half-dozen.

The investigators were able not only to simultaneously categorize more immune cell types than ever before seen at once but, at the same time, to peer inside those cells and learn how various internal processes differed from one cell type to the next.

"We can tell not only what kind of cell it is, but essentially what it's thinking, what it's been doing, and what it may soon do or become," said Garry Nolan, PhD, professor of microbiology and immunology and the senior author of the study detailing the advance, to be published May 6 in *Science*.

With this new approach, the scientists were further able to show the unexpected effects of a drug recently approved for treating certain leukemias — dasatinib — on biochemical activities taking place inside various types of cells, offering a possible explanation for some of dasatinib's side effects as well as suggesting potential new uses for the drug.

In the study, Nolan and his colleagues simultaneously monitored 34 different substances found inside and on the surface of different cell types produced in human bone marrow, the place where all immune and blood cells, as well as blood disorders such as leukemia, originate.

By measuring large numbers of cell features all at once with the new technology — called mass cytometry — the team could capture subtle transitions between cell states in, essentially, a high-resolution snapshot of the entire blood-forming system, he said. Scientists normally think of the blood and immune cells as differentiating in a series of discrete steps. However, the authors showed that the transitions from one cell state to another are marked by gradually shifting levels of cell-surface markers and varying amounts and activation states of several intercellular molecules.

Mass cytometry builds on an established technology known as fluorescence-activated cell sorting, or FACS, which is in widespread use throughout the world. FACS was developed in the laboratory of Leonard Herzenberg, PhD, professor emeritus of genetics, under whose direction Nolan did his PhD work in the 1980s.

Both FACS and mass cytometry employ antibodies to specifically tag particular surface features on cells.

With traditional FACS, antibodies are designed to tag diverse cell features. Then the antibodies are affixed to differently fluorescent dyes that color-code these antibodies according to which cell feature they target. After being bathed in these antibody-dye preparations, cells are passed single-file through a tube and stimulated by laser pulses, which cause the dye molecules to give off bursts of light. Different wavelengths of light emitted by the dyes correspond to the cellular features the dyes have tagged. FACS technology, though over 30 years old, is a mainstay of immune studies, as well as cancer and vaccine research.

But researchers are eager to squeeze ever more information out of each cell they examine. This requires examining ever more cell features at once, and there are only so many colors in the rainbow. The ability of FACS to distinguish between any more than a half-dozen dyes is constrained by those dyes' overlapping fluorescence patterns.

Three years ago, Nolan was approached by Scott Tanner, a physical chemist now at the University of Toronto.

"He buttonholed me at a meeting," said Nolan, laughing. "I was trying to get away from him, but after he'd been talking for a few minutes I realized this was something I'd better start paying attention to. He clearly had something that, if true, was revolutionary in its potential."

Tanner's team was adapting for biological purposes an existing instrument that is typically used for gauging precise levels of added rare-earth impurities in semiconductors and for geological purposes. The new instrument, called a mass cytometer, promised to more than double the number of molecular features that could be measured simultaneously in each cell. Nolan, realizing that such an instrument could be used to learn much more about the immune system and cancer stem cells, was eager to bring his group's expertise to bear on its development. The Stanford team has worked in close collaboration with the new instrument's developers ever since.

Instead of dyes, mass cytometry joins rare-earth metals to antibodies, which in turn detect cellular features and processes. "The rare earths are a series of 17 elements, mostly at the bottom of periodic table, that nobody wanted to learn about in chemistry class, myself included," said Sean Bendall, PhD, a postdoctoral researcher in Nolan's lab. However, these elements turn out to particularly useful for biological applications, said Bendall, who shared first authorship of the *Science* paper with Erin Simonds, a graduate student in Nolan's lab.

"They're not all that rare in nature, but they're normally never found in the body," Bendall said. "If I looked at a sample of your blood and found some europium or ytterbium or neodymium in it, I'd say you were in deep trouble." So rare-earth elements stand out in a crowd.

What's more, these elements can be subdivided into as many as 100 variants with distinct atomic weights. Mass cytometry can easily detect those differences. "We need relatively few rare-earth atoms per cell for our instrument to see them," said Bendall.

In mass cytometry, cells are paraded one by one through a tube and sprayed into a tiny chamber in which they are heated to about 13,000 degrees Fahrenheit and vaporized into successive clouds of atomic nuclei

and loose electrons. Next, the contents of each cloud that was once a cell are essentially flung against a wall with equal force. The lightest atoms arrive first, then the next-lightest and so forth. A detector counts the atoms as they land, and from this the instrument can determine their mass. The mass tallies how many copies of each metal-tagged antibody were stuck to the cell and, therefore, how many copies of each molecular feature were present on, or in, the cell in the first place.

In the *Science* study, Nolan and his colleagues used the instrument to simultaneously monitor 13 separate molecular features on the surfaces of cells in samples taken from two healthy humans' bone marrow, and classified the cells into numerous distinct categories. The investigators simultaneously monitored activation states of 18 different intracellular protein targets. Protein activation levels give important clues about particular cellular decisions that have been or can be made, such as whether a cell is about to divide.

"As a prelude to looking at leukemic bone-marrow samples down the road, we wanted to first characterize the cells in normal bone marrow to see how their behaviors change as they mature," said Simonds.

The Nolan group perturbed cells by exposing them to various substances, including signaling molecules that sometimes circulate in our own blood, as well as foreign materials such as fragments of bacterial cell walls that are known to excite immune responses. "In essence," said Nolan, "we are interviewing or interrogating the cells, forcing them to reveal their inner thought processes." Some of these stimulatory tests were done in the presence of dasatinib, a drug used to treat chronic myelogenous leukemia and certain cases of acute lymphoblastic leukemia. Dasatinib is in clinical trials for several other indications, including some solid cancers.

When the Nolan group used a chemical, pervanadate, to "release the

brakes" on a universal pro-cell-survival behavior, dasatinib blocked action in every cell type except one, the immune sentinels called dendritic cells. Simonds said this new finding demonstrates mass cytometry's capacity to ferret out tiny differences in cellular behavior that may help explain drugs' side effects as well as to indicate potential new uses for existing drugs.

The more measurements your tailor makes, the better the fit. It's the same with cell biology. "Our entire lab has already shifted from fluorescence-based measurements of cell features to this new MS-based method, because we get a much more complete picture," said Bendall.

Nolan has reported that he owns stock in the company Tanner created to develop and market the new system.

Provided by Stanford University Medical Center