# New way to sort cells without limitations of traditional methods

March 31 2015, by Christopher Vaughan

A team of Stanford University School of Medicine researchers has come up with a new way of analyzing individual cell types by applying advanced mathematical analysis to the cells' contents.

The method is analogous to analyzing a smoothie to find what fruits went into making it, the researchers say. A paper describing the method, called Cibersort, was published online March 30 in *Nature Methods*.

Analyzing and sorting individual cells according to the proteins they display on their surfaces is an essential part of stem cell science and cancer research. By analyzing these proteins, known as cell surface markers, scientists can figure out what kind of cells they are dealing with and see how the cell samples, taken from an animal over a period of time, change.

With cancer, the presence or absence of certain cell markers can make a huge difference in a patient's prognosis and what treatments will be most effective.

But many kinds of tissue can't be analyzed easily or accurately using current methods of cell sorting, hampering scientists' ability to do research and clinicians' ability to find the most effective therapies for cancer and other diseases.

"The basic problem is that we often want to count cell populations in tissues, but we rely on methods that require tissues to be collected and

stored, then separated into individual cells or sliced into sections, and then labeled with antibodies to specific markers," said Ash Alizadeh, MD, PhD, assistant professor of medicine and the senior author of the paper. "Each of these steps has limitations."

## How cells are sorted

Decades ago, Stanford researchers were among the world's leaders in developing a method for analyzing cells called flow cytometry. With this method, tissues are first separated into individual cells and exposed to fluorescently labeled antibodies that attach to particular cell surface markers. Then a few cells at a time, in tiny drops of water, are passed in front of a laser that excites the fluorescent antibodies and an optic sensor that counts each type of cell in the drop. In some machines, the different kinds of cells can be sorted into various containers.

The standard method of cell sorting requires breaking up tissues, or disaggregating them, into individual cells, Alizadeh said. This is a rough process that destroys certain cell types and renders some tissues useless for study. In addition, the traditional method of preserving medical samples makes it impossible to process them in this way, he noted. Also, fluorescently labeled antibodies must be produced for each specific cell protein in which the scientists are interested. Antibodies may not be available for some proteins, he said.

The solution the researchers came up with is to sort not the cells, but their contents. "We were asking, 'Can you take a tissue, blend it up, look at the contents and tell what kinds of cells they came from?'" Alizadeh said.

## Reconstructing the cellular 'smoothie'

In developing the new method, Alizadeh and his colleagues focused not on the protein cell surface markers, but on the RNA on which those proteins were patterned. Postdoctoral scholar Aaron Newman, PhD, devised a computer algorithm to reconstruct the type and number of original cells based on the RNA contents of the mixture of all the cells.

"It's like reconstructing a smoothie," said Newman, a lead author of the paper. "You know it has a lot of different kinds of fruit in it, but you don't know right away how many of each type. However, you might know that strawberries had a certain amount of sugar and red coloring, while oranges have a different amount of sugar, orange coloring and more tartness. If you analyze each of these qualities, you can reconstruct how many of each kind of fruit went into making the smoothie."

In addition to avoiding the problems inherent in breaking up tissues into single cells, researchers using this method won't need fluorescently labeled antibodies for the cell surface markers they are looking for, he said.

Some of the most exciting recent advances in the treatment of cancer involve the use of novel drugs that engage immune responses in patients to fight the disease. These drugs often target rare and dormant populations of immune cells that reside within tumors. While some of these drugs can be dramatically effective for patients with very different tumor types, not every patient benefits equally, and some tumor types appear not to respond to these new immune therapies.

"A significant, ongoing effort is to find which immune cells mediate response and resistance to these drugs, to allow their more directed and precise use in a personalized fashion," said Alizadeh, who is also a member of the Stanford Institute for Stem Cell Biology and Regenerative Medicine and the Stanford Cancer Institute. "If we apply Cibersort to cancer tissues, we think we will be able to see amazing

things."

## Targeting cancer treatments

If the researchers apply Cibersort to old tumor samples from patients whose clinical history is known, they may be able to learn what kinds of cells signal more or less deadly cancers. They may also learn what kinds of treatments work better or worse in various subtypes of cancer. This sort of information might be most important for the antibody cancer therapies.

"There are early hints that it is very important to know about the presence of specific types of immune cells in the tumor before and after certain therapies are given, and how those cells change over time," Alizadeh said.

**More information:** Robust enumeration of cell subsets from tissue expression profiles, DOI: 10.1038/nmeth.3337

Provided by Stanford University Medical Center