SASCA tackles single drug-resistant cancer cells

It began with a peculiar observation: in 2004, Paul Li and colleagues at Simon Fraser University (Canada) had designed a microfluidic device with 3D flow control that could capture and manipulate single cells (Anal. Chem. 2004, 76, 5282–5292). While doing the experiments, the investigators noticed that the single yeast cells seemed to spit out the fluorescent marker they were dosed with, causing the fluorescence to decrease.

The observation prompted the investigators to think about how they could turn the spitting-out phenomenon, known as efflux, into something practical that met a clinical need. In their latest AC paper (2008, 80, 4095–4102), Paul Li, Xiujun Li, and Victor Ling describe how they use the microfluidic device to measure drug efflux and the inhibition of efflux in multi-drug-resistant leukemia cells. “We envision this will [eventually] help the administration of chemotherapy, by knowing what kind of efflux process is occurring in a patient’s cancer cells and which inhibitor will reverse the process,” says Paul Li.

Multidrug resistance occurs when cancer cells barricade themselves against a variety of drugs; it is the bane of chemotherapy. One way cells resist is by pumping out drugs. The membrane-bound, energy-dependent P-glycoprotein (Pgp) is a protein that does the pumping and tends to be overexpressed in cancer cells. Inhibitors can stop Pgp from functioning, but clinicians need to fiddle around with them to come up with the magic combination of drug and inhibitor for effective therapy.

Methods exist to measure multidrug resistance, but they have drawbacks. Flow cytometry requires thousands of cells for the analysis, a requirement that’s sometimes hard to meet when precious few cells are at hand. To circumvent the sample size issue, a single-cell microfluidic analysis technique, called different-single-cell analysis (DISCA), has been developed to work with a limited number of cells.

Paul Li and colleagues attempted to use DISCA but ran into problems. “We had a hard time observing what we expected to see,” says Paul Li. “The cells came from the same batch of cultured cells, but they had different properties.”

Because individual cells differ in their levels of Pgp expression—and thus in their extent of drug resistance—the investigators struggled to compare the behavior of control and experimental cells. To overcome the comparison problem, Paul Li and colleagues decided to use the same cell for both the control and the experiment. They bestowed the name SASCA, for same-single-cell analysis, on their approach.

The first step in the SASCA process is the control experiment—accumulation of a drug in a cell followed by the measurement of its efflux without an inhibitor. For the experimental step, drug accumulation is repeated in the same cell, but this time, the efflux is measured in the presence of an inhibitor. “We see the results clearly,” says Paul Li, who notes that much of the work was done by his graduate student, Xiujun Li. “If the cell has low multidrug resistance, then we see a little bit of a reversal with the inhibitor. In the case of high multidrug resistance of a cell, we see a much better and greater reversal effect” with the inhibitor.

Once they were convinced that SASCA worked well, the investigators tested two compounds from traditional Chinese herbal medicine for the ability to inhibit drug efflux. Isoliquiritigenin is derived from Chinese licorice; by the SASCA method, it didn’t demonstrate any inhibition of drug efflux. Sodium artesunate is an antimalarial drug suspected of having anticancer properties. Paul Li had come across the compound while on a sabbatical leave in Germany, “so I thought we’d try it. We found that it indeed showed a drug reversal effect. We were so excited about it! There was some preliminary work done on the drug, but it wasn’t conclusive. But with our SASCA method, we see very clearly that this drug shows the reversal effect.”

Besides further engineering the microfluidic device to handle multiple cells at once, Paul Li wants to use cells from individual cancer patients. “I hope this method will eventually be used to provide prognosis information,” he says. The idea is to let clinicians determine the appropriate combinations of drugs and inhibitors by the SASCA method to figure out the best course of action. Paul Li says, “We’re providing a tool to do a functional assay that better implements chemotherapy.”

—Rajendrani Mukhopadhyay