Chemists devise inexpensive, benchtop method for marking and selecting cells

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Chemists at The Scripps Research Institute (TSRI) have found an easier way to perform one of the most fundamental tasks in molecular biology. Their new method allows scientists to add a marker to certain cells, so that these cells may be easily located and/or selected out from a larger cell population.

The technique, which is described in a recent issue of the chemistry journal Angewandte Chemie International Edition, makes use of the tight binding of two proteins that are cheaply obtainable but are not found in human or other mammalian cells. As such, it has advantages over existing cell-marking techniques.

"This new technique is cheap, easy and sensitive," said TSRI Institute Professor Richard A. Lerner, who is the senior author of the new report. "The method should be useful in a variety of applications that require separating out certain types of cells."

Looking for a Better Way

The best-known cell marker in use today is GFP (green fluorescent protein), a jellyfish protein that emits a distinctive green light when illuminated by certain other light wavelengths. When scientists want to add a new gene to cells, for example to produce a therapeutic protein, they often construct a genetic sequence that also includes the GFP gene. Thus the cells that successfully produce the new protein will also

produce GFP, whose fluorescence allows these cells to be identified and even sorted out from a larger population.

But fluorescence-based cell sorting is relatively expensive and cumbersome. Alternative cell-marking techniques use marker molecules to which antibodies or metals will bind tightly, but these are apt to have unwanted side effects on the cells that they mark. Lerner's team, led by first author Yingjie Peng, a postdoctoral fellow, set out to invent a better method.

The new method exploits a special property of chitinase enzymes, which evolved to break down chitin—a tough, sugar-derived material found, for example, in crab shells, squid beaks and the cell walls of fungi. In addition to a main chitin-breaking domain, chitinases have another active structure, a "chitin binding domain" (ChBD). "It makes a super-strong bond with chitin," said Peng. In recent years, scientists have begun to use this high-affinity binding of ChBD and chitin as a marker system, typically for selecting ChBD-tagged proteins in a lab dish. The new method uses ChBD to mark and select cells.

A Powerful Tool

In the basic technique, a new gene can be added to cells within a larger DNA vector that also includes the genetic sequences for ChBD and GFP. The ChBD molecule will be produced in such a way that it ends up being held on the outer surface of its host cell's plasma membrane—and the GFP molecule will sit just inside the membrane. The GFP serves as a visual beacon, while the ChBD serves as a handy gripping point for cell selection.

After exposing a culture of test cells to this experimental ChBD-containing vector, the scientists was able to see, via the GFP tags, which cells were expressing them, and was able to select them out easily, with

high sensitivity, using magnetic beads coated with chitin. "This is a relatively easy benchtop method," Peng said. Importantly, these selected cells could produce progeny cells that seemed normal and healthy.

Because the ChBD marker, in the vector, is produced in a way that anchors it to a cell's membrane, it also can serve as a powerful tool for selecting just the membrane fraction of a sample of cellular material. Peng and his colleagues demonstrated this using chitin beads to quickly isolate a pure fraction of membrane material from ChBD-marked test cells.

Cellulase enzymes, which break down the ubiquitous plant compound cellulose, also have a high-affinity cellulase-binding domain, which can be employed in the same way as the ChBD.

The scientists expect that the new cell-marking method will help to streamline another major molecular biology technique, which was pioneered by the Lerner laboratory in parallel with the group of Sir Gregory Winter at the Laboratory of Molecular Biology in Britain. This technique allows scientists to produce very large and diverse libraries of antibody arms, and to sift through them, or "pan"—as gold miners pan for nuggets—for those that might be of use, for example in therapies. ChBD-based markers should be useful in boosting the efficiency of this panning process, said Peng.

The Lerner laboratory is also investigating the potential use of ChBD-based cell marking in living animals, for example to track the fates of selected cell types throughout an animal's lifespan.

More information: "Engineering Cell Surfaces for Orthogonal Selectability," onlinelibrary.wiley.com/doi/10 … e.201201844/abstract

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