Tool Detects Protein Variations May Benefit Cancer Research

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For decades, researchers have analyzed proteins in cells with a laboratory technique called Western blotting. But this method couldn't provide a complete picture when researchers wanted to study important differences at the single-cell level. In cancerous tumors, for example, heterogeneity exists even in small populations of cells. Because conventional Western blotting uses bulk measurements of cell groups, it can't reveal vital details about protein variations.

That's changed, thanks to the work of Amy Herr, Ph.D., and her colleagues at UC Berkeley's bioengineering department. They developed an analysis tool called single-cell Western (scWestern) blotting. Unlike conventional Western blotting which provides results as averages, scWestern blotting can analyze proteins for more than 1,000 individual cells simultaneously and identify subpopulations of cells.

> *By providing a detailed view of heterogeneity, this tool can lead to a better understanding of cancer biology and may facilitate discoveries that improve cancer treatment.*

Developed in 1979, the conventional Western blot process requires several steps. After the cell sample is placed in a gel, proteins are separated with the use of electrophoresis (which allows an electric field to sort molecules by their size). The proteins are transferred out of the gel to a polymer membrane. In the final step, researchers identify specific proteins by exposing them to antibodies that bind to those target proteins (the name Western blotting is a play on words — it's a nod to Southern blotting, the DNA detection method named after its inventor, Edwin Southern).

That approach has some notable shortcomings: It's a time-consuming, labor-intensive process, and it can't detect proteins at the single-cell level. When Amy Herr arrived at Berkeley's bioengineering department in 2007, she was determined to find a better research tool. "Each cell is packed with biomolecules that are telling it what to do," she says. Among other things, that affects whether cancers develop aggressively or not. "How do we get inside that cell to see what's happening?" says Herr. "And how do we do that, not just for one of these tiny little packets of really dynamic information, but for thousands of them?"

Significant innovations did exist for single-cell detection of RNA and DNA, but not for proteins. That matters, says Herr, because proteins more accurately reflect the variations underlying cell behavior. Essentially the molecular machines of the cell, proteins can both indicate and cause differences in cell behaviors. "There are all kinds of things that happen in proteins, like chemical modifications, and none of that activity is captured in measurement of DNA or RNA," she says.

To address that, Herr turned to a field she'd worked in for a decade: Microfluidics. It involves the design or use of tools that control the movement of fluid through microscopic channels. In 2010, Herr received an important award from the National Institutes of Health, to help fund her development of a microfluidics tool that could detect proteins at the single cell level. Called the New Innovator Awards, it provides $1.5 million over five years and is intended for young researchers who take bold approaches to biomedical challenges. Says Herr:
"It's a once-in-a-lifetime chance from the federal government, recognizing we need to unleash creativity to allow people to do things that are truly high-risk."

It proved to be a worthwhile investment, helping Herr and her lab researchers design the scWestern blotting system. With this tool, the cell sample is captured in an array of microwells that are fabricated into a thin hydrogel layer. The size of the microwells and the thickness of the hydrogel layer are both about half the diameter of a human hair. Like traditional Western blotting, it uses electrophoresis to separate proteins. But scWestern eliminates the time-consuming step of transferring the proteins a separate membrane. That's because the gel in the channel contains a molecule (benzophenone) that reacts with UV light. When the gel is exposed to UV light, the benzophenone binds with the proteins and locks them in place. The proteins are then exposed to antibodies that bind with target proteins and allow them to be identified.

With scWestern blotting, researchers can detect up to four specific proteins in about 1,000 individual cells at once. Herr says that although other research tools allow direct measurement of proteins, they have significant limitations. While those tools can only detect whether a protein is present, scWestern blotting can also identify proteins by size. That means It can measure modifications or chemical changes to those proteins by identifying differences in molecular

mass. “That’s an important contribution we’ve made,” she says. Such detailed detection could allow better experiments for cancer research and reveal why some tumors don’t respond to chemotherapy, for example.

The first patents for the scWestern blotting system were filed in 2011 by UC Berkeley's Office of Technology Licensing [http://ipira.berkeley.edu/]. The next year, Herr received additional commercialization support from the university — she was selected for UC Bekeley’s Bakar Fellows Program. Designed to help Berkeley innovations reach the marketplace, the program provides funding for research fellows as well as networking and mentoring services. Herr soon decided the best commercialization path would entail launching a company. “We had several established companies interested. I guess they didn’t move fast enough,” she says. “It came down to timing."

For co-founders, she turned to Kelly Gardner, Ph.D. (who became CEO) and Josh Molho, Ph.D. (who became CTO). Gardner worked previously in Herr’s research group, although not specifically on scWestern blotting. And Herr had known Molho since the two met in graduate school at Stanford University. "I know Kelly and Josh so well. There are ups and downs, points where hard decisions had to be made, and working with a team you inherently trust is the biggest thing when you’re founding a company."

In December 2013, they formed Zephyrus Biosciences, and Berkeley's Office of Technology Licensing (OTL) granted a letter of intent agreement for licensing. That provided several months of exclusivity for the company, which was crucial for a successful launch, says Molho. "We had to have a clear path to licensing, or the investors wouldn't be interested,” he says. “In the life sciences space, there's a lot of weight put on intellectual property. If you don't have any, it's generally assumed that somebody else will come along and eat your lunch.” Herr agrees that the OTL’s support played a vital role. "Having them there every step of the way has helped us to be able to do much more than we could alone,” she says. After about a month of negotiation, Zephyrus received an exclusive license in July 2014.

“Not only do they have a technology that solves a need, but the team really leveraged the resources available to them,” says Terri Caron-Sale, Senior Licensing Officer at UC Berkeley’s OTL. That included participating in a 10-week workshop called Lean Launchpad, organized and sponsored by the UC San Francisco Innovation, Technology & Alliances (ITA) [https://ita.ucsf.edu/] The lean startup approach emphasizes rapid innovation on a small budget. Historically, those techniques were mainly applied to the software industry, but the Lean Launchpad workshop showed how life sciences companies could be lean startups too. As part of that workshop, the founders interviewed more than 100 potential customers, which helped the company refine its target market, says Molho.

By September 2014, Zephyrus had received a $350,000 Small Business Innovation Research grant from the National Institutes of Health, and had raised $1.5 million in funding (led by angel investor group Life Sciences Angels). In the following months, the founders expanded their laboratory, hired a small team, developed instrumentation and microchip prototypes, and pitched venture capitalists for follow-on investment. They also discussed possible strategic investments or partnerships with more than 10 large companies. In March 2016, discussions with Bio-Techne Corp. [https://www.bio-techne.com/] became more than just talk. The Minneapolis-based life-sciences company (with $499 million net sales in fiscal 2016) acquired Zephyrus, which had six full-time employees and a team of consultants at the time. A few months after the acquisition, Zephyrus CEO Gardner was included in MIT Technology Review’s annual “35 Innovators Under 35” list. Says Molho: “We formally founded the company at the end of 2013, we didn't have a lab until April 2014, and the company was acquired a little less than two years later. That's a somewhat unusual path for a life science start-up.”

At Bio-Techne, the scWestern tool is branded as Milo (http://www.proteinsimple.com/milo.html) and became commercially available in 2016, says Molho (currently director of engineering for Milo).

"I'm a mechanical engineer, and I could work in lots of different areas," he says. "But I like life sciences because with the end goal, you're either increasing scientific knowledge or you're impacting healthcare." Regarding the impact that scWestern blotting could have, Molho notes there are several research areas — like cancer and stem cell research— where scientists are seeking more detail about cell populations that aren't homogenous. "They're asking questions like, what's different about this sub-population of tumor cells that isn't getting killed off by a drug? Or they have stem cells that they want to turn into heart cells, and want to understand why some of those stem cells aren't transitioning," says Molho. By revealing more about the proteins in those cells, scWestern may help answer those questions. Says Molho: "It's kind of a long game, but I hope that with this technology — in its current and other forms that may come in the future — we'll play a small role in helping improve outcomes for people."

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