“Let the Cells Tell the Story”

New tech is giving scientists unprecedented insights into single cells’ inner workings.

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Kelly Paulson, MD, PhD, a Fred Hutchinson Cancer Research Center postdoctoral fellow in the Chapuis Lab, was on the hunt. She was tracking her quarry’s traces and the devastation it left behind. But it stayed just beyond her grasp.

Paulson was trying to find the source of a cancer recurrence. The patient’s rare, aggressive skin cancer had responded to immunotherapy for 22 months — and then suddenly, inexplicably, it returned.

But why? Paulson checked the obvious boxes. Mutations in the tumor? Didn’t explain it. The experimental immune cells that her mentor, Aude Chapuis, MD, engineered to attack the cancer? Still there. Nothing she saw could explain why, after nearly two years, that immunotherapy no longer held the cancer at bay.

She needed to look at the inner workings of the individual cells involved, both tumor and nontumor, to understand what was happening. But standard approaches could only paint her a blurry picture.

“Using our previous technology, we looked at everything all mixed together,” she explained. “If you think about a blender, if we had dropped blueberries and strawberries and bananas all in the blender, we would push the button and blend it, and we’d see a smoothie. When you’re looking at a smoothie, it’s nearly impossible to determine if there was a problem with a single strawberry.”

Instead, she needed to see the individual components: the different cancer and immune cells, and the many other noncancerous cells that could be influencing the course of the patient’s disease.

So Paulson tried a new approach. She and Chapuis teamed up with Hutch colleagues Jason Bielas, PhD, a cancer geneticist, biostatisticians Raphael Gottardo, PhD, and Valentin Voillet, PhD, and University of Washington skin cancer researchers Paul Nghiem, MD, PhD, and Shailender Bhatia, MD, to apply a new technology called high-throughput single-cell RNA sequencing. It gave them insight into the genes that individual cells were — or were not — using to function. She compared blood samples taken from the patient before and after treatment.

Immediately, the picture snapped into focus.

“With this new technology, we could instantly see that the tumor was what was escaping in this particular cancer,” said Paulson. The tumors had made themselves invisible to the engineered immune cells by tuning down a key protein that made their target visible on the cell surface — all without needing to change even a letter of their genetic code.

That’s the beauty of high-throughput single-cell RNA sequencing technology, said Bielas, who helped bring the new technology that Paulson used to the Hutch: “You can let the cells tell you the story,” he said.

Single cells build a bigger picture

When Paulson peered inside the patient’s individual cells, she wasn’t interested in their DNA. A cell’s genetic code doesn’t tell you how what a cell is up to, just like a car’s blueprint can’t tell you how fast it’s going or what direction it’s turning. In a cell, the information about what it’s doing, how it’s behaving, comes from a molecule known as messenger RNA.

Cells copy the genes they need to use into messenger RNA molecules, which are then used as a recipe for making proteins. To work properly, a specific cell needs many copies of certain proteins, fewer copies of others, and no copies of still others. A snapshot of the types and numbers of different mRNA molecules present gives scientists a window into what cells are doing at any given time.

But getting a snapshot of the mRNA spectrum of many thousands or millions of cells takes some doing. High-throughput techniques — in which enormous numbers of cells can be analyzed in a single experiment — have only been around a few years, but are already transforming the questions that researchers can ask.

How to see a single cell

There are two general approaches to single-cell RNA sequencing. Both rely on molecular barcodes that ensure all the mRNAs from a specific cell share a unique tag.

One method separates individual cells from each other and adds a unique barcode to the mRNA in each cell. This is the approach used in the 10X Genomics platform that Bielas helped validate and Paulson used.

In an instrument-free, “split-pool” approach, each cell’s unique barcode is built in a stepwise process. Cells are split into groups, and each group gets its own tag. Then the cells are shuffled, regrouped, and tagged anew. This is repeated enough times, using enough pools, to build a unique barcode for each cell.

“It’s kind of like playing the lottery; every time you pick a number — say it’s between zero and 99 — it’s not unique. But once you’ve done this six times, for example, your number is going to be different than all the other numbers,” said Georg Seelig, PhD, a University of Washington bioengineer whose group has developed their own experimental split-pool–based method, SPLiT-seq. “At the end, you have more barcode combinations than you have cells.”

Finding the unknown unknown

When Paulson and Chapuis were hunting down the key change in her patient's cancer cells, they didn't know what they were looking for or in which cell population they would find it. One of the great benefits of single-cell RNA sequencing — and what sets it apart from many other experimental tools — is the fact that researchers can use it to detect differences between cells or even entirely new cell populations that they didn't know existed before, said Bielas.

Hutch's Anthony Rongvaux, PhD, studies how cancer metastasizes, or spreads through the body, particularly in the skin cancer melanoma. Like Chapuis and Paulson, his lab took advantage of high-throughput, single-cell RNA sequencing to go looking for an unknown population of cells that help promote metastasis.

He specifically focuses on macrophages, a type of immune cell. Though macrophages are best known as the immune system's garbage disposals, hoovering up infected cells, they can sometimes act to tamp down an immune response. Mounting evidence suggests that there are many more subtypes of macrophage than initially recognized, and tumors often find ways of turning macrophages from cancer-fighters into cancer-promoters that can even block other immune cells from attacking the tumor.

What distinguishes these different types of macrophages isn't their DNA, it's what they're doing. And those differences can be found in their mRNA.

Rongvaux's group teamed up with Bielas and Gottardo to use high-throughput single-cell RNA sequencing to "let the macrophages tell us who they are," he said.

Led by Voillet and the Rongvaux Lab's Trisha Sippel, PhD, the scientists found a previously undescribed population of macrophages that have been turned into tumor-helpers. Paulson, who also contributed, dubbed them "bad-o-phages." These turncoats use a distinct set of mRNAs, which gives researchers clues about how the bad-o-phages survive within tumors and how they might be thwarting immune cells called T cells.

Exploring the ecology of cancer

Jerry Radich, MD, a Hutch physician-scientist who studies leukemia and worked with Bielas to validate the 10x platform, studies leukemia and its treatment — who relapses and who is cured. But the answers to these questions may lie beyond the cancer cells themselves.

"Cancer is an ecosystem with many [genetic] clones that may compete or cooperate with each other," to say nothing of the other cells, like immune cells that interact with cancer cells, said Radich. He and a Hutch colleague, Amy Paguirigan, PhD, were among the first to examine the genetics of leukemia at the single-cell level, describing a much more complex evolutionary tree for the cancer than had previously been appreciated.

With the arrival of the new single-cell RNA sequencing tech, Radich and countless other researchers are rushing to put it to use. A greater ability to understand all the components of a

cancer ecosystem — cancerous and noncancerous — and how the ecosystem changes after therapy will help scientists advance and improve therapies.

Take certain cell-based immunotherapies in which T cells are engineered to target tumor cells: Some patients' disease vanishes, but some patients' disease resists or recurs.

"You could infer things about function from RNA expression that would suggest that 'Oh, yeah, in this population of people who are cured, those T cells are really revved up and attacking the tumor,'" said Radich. In contrast, perhaps the T cells in patients whose cancer doesn't respond also have distinguishing patterns of mRNA: "You could say, 'Look at these lazy bums, they're not pulling their weight.'"

Hutch's Mark Headley, PhD, studies how the immune system contributes to cancer's spread. Focusing specifically on metastatic tumors that arise in the lung, he zeroes in on the earliest moments of metastasis, when a "small scrum" of immune cells are interacting with perhaps just one metastatic tumor cell.

"My viewpoint is, if you want to understand the process [of metastasis], you really have to zero down on the single-cell level," said Headley. The intercellular communication between immune cells and metastatic tumor cells is setting the stage for the future, Headley believes, both determining which metastatic tumor cells succeeds in seeding a new tumor and also helping shape the long-term tumor microenvironment.

Translating insights into the clinic

Single-cell sequencing technologies are already providing insights that could make their way into the clinic. Paulson, with Chapuis and her team member Megan McAfee, PhD, is working to improve treatments for other cancer patients.

She works with patients who have Merkel cell carcinoma, or MCC, a rare and aggressive skin cancer. Immunotherapies have transformed care for these patients, but not everyone's tumor responds. The cells most responsible for eliminating tumors are T cells, which rely on a unique molecule called the T-cell receptor, or TCR. Genetic engineering has made it possible for scientists to make other patient's T cells express the TCR from a potent anti-tumor T cell. With single-cell RNA sequencing technology, Fred Hutch researchers can find those TCRs more easily than ever.

A field in flux

Because the field is so young, scientists are still trying to determine which platform best suits their individual research needs, said Headley. He uses both the 10X platform and collaborates with Seelig to apply his split-pool approach. Each method is somewhat different in its requirements for how cells are handled and in the data it produces. Headley is exploring the contexts in which each approach works best.

Moderately high-throughput single-cell RNA sequencing techniques were first published in 2009, but the field really ramped up in 2015, when new methods, called Cyto-seq, Drop-seq and InDrop,

made it possible to sequence RNA from tens or hundreds of thousands of cells in a single experiment.

The sheer size of the datasets generated by these techniques are driving their own series of advances in data science as computational biologists develop better ways to analyze, visualize, store and access these data.

Trying to take advantage of the new technologies without taking into account how the data will be generated and analyzed can lead to wasted experiments, said Gottardo, who leads Fred Hutch's Translational Data Science Integrated Research Center and holds the J. Orin Edson Foundation Endowed Chair.

Close collaborations with computational biologists ensure that researchers design experiments so that clear biological questions can be answered, he said.

In the future, Radich expects that technologies will make it possible to combine DNA sequencing and RNA sequencing from the same cell, allowing scientists like him to directly link functional, RNA-based information to a cell's unique genetics.

While more applications await over the horizon, Paulson is jumping on the opportunities she sees for single-cell RNA sequencing technologies to advance cancer immunotherapy.

"I think they're really revolutionizing the field. They'll be useful for a lot of different applications," said Paulson.

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