



Sidi Chen

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NEW TOOLS APPLIED TO OLD PROBLEMS

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Sidi Chen, PhD, Assistant Professor of Genetics, is always looking for practical, more efficient solutions to familiar problems. When dissatisfied with a tool, for instance, he and his lab invent a better one. He has recently used this approach to make advances in fundamental genetics, genetically-engineered immune cells, and the biology of metastasis.

Fundamental genetics is a logical place to begin. In the February issue of *Cell Systems*, Dr. Chen and colleagues, most of them from his lab in the Systems Biology Institute on Yale West Campus, published the results of their extensive search for genes that either incite cancer cells or help them evade detection by the immune system. Both types are implicated in tumor growth.

Using the genetic editing tool CRISPR and mice models, they screened more than 20,000 genes through 80,000 sgRNA constructions. A dozen genes stood out as enablers of tumor growth. The champion that sparked the most aggressive tumors was also, until now, the most obscure—a gene called Prkar1a. It caused rampant growth by its absence. When the scientists removed Prkar1a from cells, tumors went crazy.

Dr. Chen explains: “The main reason patients get cancer is that some cells acquire mutations. A mutation can make a gene super active and drive cell proliferation, like if your gas pedal gets stuck. That’s an oncogene. The opposite is a tumor suppressor gene. If a mutation destroys the function of this gene, you lose the power to suppress tumor growth, meaning you lose your brakes. Losing a tumor suppressor is at least as important as gaining an oncogene. Prkara is a tumor suppressor.”

Tumors without Prkar1a not only grew more rapidly, their cancer cells corrupted the host’s innate immune system, tricking immune cells into accelerating tumor growth instead of fighting it.

If removing the gene speeds up growth, why not boost immunity by somehow saturating cells with the suppressor Prkar1a? “That’s very difficult,” said Dr. Chen. “Taking something out is easy, but if the cancer cell has already lost an important tumor suppressor like Prkar1a, it’s really hard to give it back. This research is for basic understanding of why cancer progresses. It’s hard to translate. That’s why I’m also working on developing new strategies for immunotherapies, meaning you get rid of the brakes on immune cells so they can fight better. Therapeutically that’s more achievable.”

One such effort is Dr. Chen’s work on chimeric antigen receptor CAR T-cells. These are T-cells that have been removed from the blood and genetically engineered to target an antigen in cancer cells. This is done by using CRISPR to insert a code in the T-cells’ genome. Then these weaponized super-Ts are

multiplied in a lab and reintroduced into the body to fight cancer. It’s a fairly new and very promising immunotherapy, but so far is approved only for B-cell non-Hodgkin’s lymphoma and B-cell acute lymphoblastic leukemia.

Dr. Chen and his lab have designed a new CAR T-cell technology that is safer, faster, more precise and efficient, more flexible and prolific, and more potent than the current platform. They call it CRISPR-Cpf1. Their paper describing it was published in March in *Nature Methods*.

“There are quite a few limitations with the current CAR T strategy,” said Dr. Chen. “That’s why we invented a new tool. It produces two, three, four times more CAR Ts than the current platform, more quickly and efficiently. We are even more excited that our tool can make more complicated CARs that recognize two antigens of the cancer cell instead of just one.”

That’s important because some patients who receive CAR Ts with only one antigen relapse after the cancer cells adapt by camouflaging the antigen to make it invisible to the CAR T. “But if the CAR T recognizes two antigens,” said Dr. Chen, “then the cancer cell has a harder problem to hide them both.”

The new tool is flexible enough to accommodate many different CAR Ts. Dr. Chen’s team is testing variations, looking for the most effective combinations against different cancers. He is particularly intent on finding something that works against solid tumors, which have proven resistant to current CAR Ts.

It’s much too early to bring this new platform to clinical trials, but Chen is confident it will reach that stage. “We think this technology will accelerate the entire process of T-cell engineering and therapeutic translation.”

His newest paper, published in April in *Nature Methods*, addresses the thorniest problem in cancer research—metastasis. “Surgeons are now so good, they can remove any primary tumor or residual tumors, for most part,” said Dr. Chen. “What kills most patients is cancer that metastasizes into vital organs like the lung, liver, brain, or upper body.”

Scientists have been investigating the complicated mechanisms of metastasis for decades. Many single genes are known to speed it up or slow it down. Dr. Chen decided to approach the problem more systematically and comprehensively. He began with the belief that metastasis is driven by the interaction of two or more genes.

“But if you have 20,000 genes,” he noted, “there are 400 million different combinations. To find and test the relevant pairs that might drive metastasis, we need two things. First, to narrow down which genes are potentially important.” He did that by focusing on mutated genes in metastasis samples.



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“Second, we need to computationally study those genes and their interactions.”

Dr. Chen’s team identified 26 genes as likely suspects, which meant 326 pairs—still lots of combinations. “We needed a way to test them all together,” said Dr. Chen. “If you lose one pair, would the cancer cells metastasize faster? Or if you lose a different pair, would that change metastasis?”

He had an idea how to get this information, but first he had to invent another new tool. This one is called MCAP (massively parallel CRISPR-Cpf1/Cas12a crRNA array profiling). Using

a library of roughly 12,000 DNA constructs, the tool allows high throughput screening of double knockout cancer cells in a mouse model. Each gene pair was targeted by 16 or more constructs.

“These cancer cells are not metastatic to begin with,” said Dr. Chen, “but if you knock out a lot of combinations and inject the cells into the mice, in some combinations each cancer cell would lose two genes, meaning they are double mutants. If you see these two genes more frequently in the metastasis in the animal, then they drive metastasis together.”

Next the team sequenced all the metastases and ranked the gene pairs according to potency. They created a map of the genetic interactions influencing the metastatic properties of the cancer cell. Lastly, they validated everything by comparing double knockouts to single knockouts to no knockouts.

Dr. Chen believes that MCAP and his findings will someday help determine if a mutation in a patient’s primary tumor is more or less likely to become metastatic. First, that prognostic information could be vital for guiding treatment. Second, identifying the drivers of metastasis could provide targets for

drug developers—perhaps the pathways to metastasis can be blocked or obstructed. Lastly, Dr. Chen thinks his tool could be used to develop immunotherapies that target metastatic cancer cells.

“Those three very different directions are enabled by this high throughput technology and what we have found so far. But we still don’t see the limit of these tools. The sky is the limit,” explained Dr. Chen. ↻