A new RNA editing tool could enhance cancer treatment

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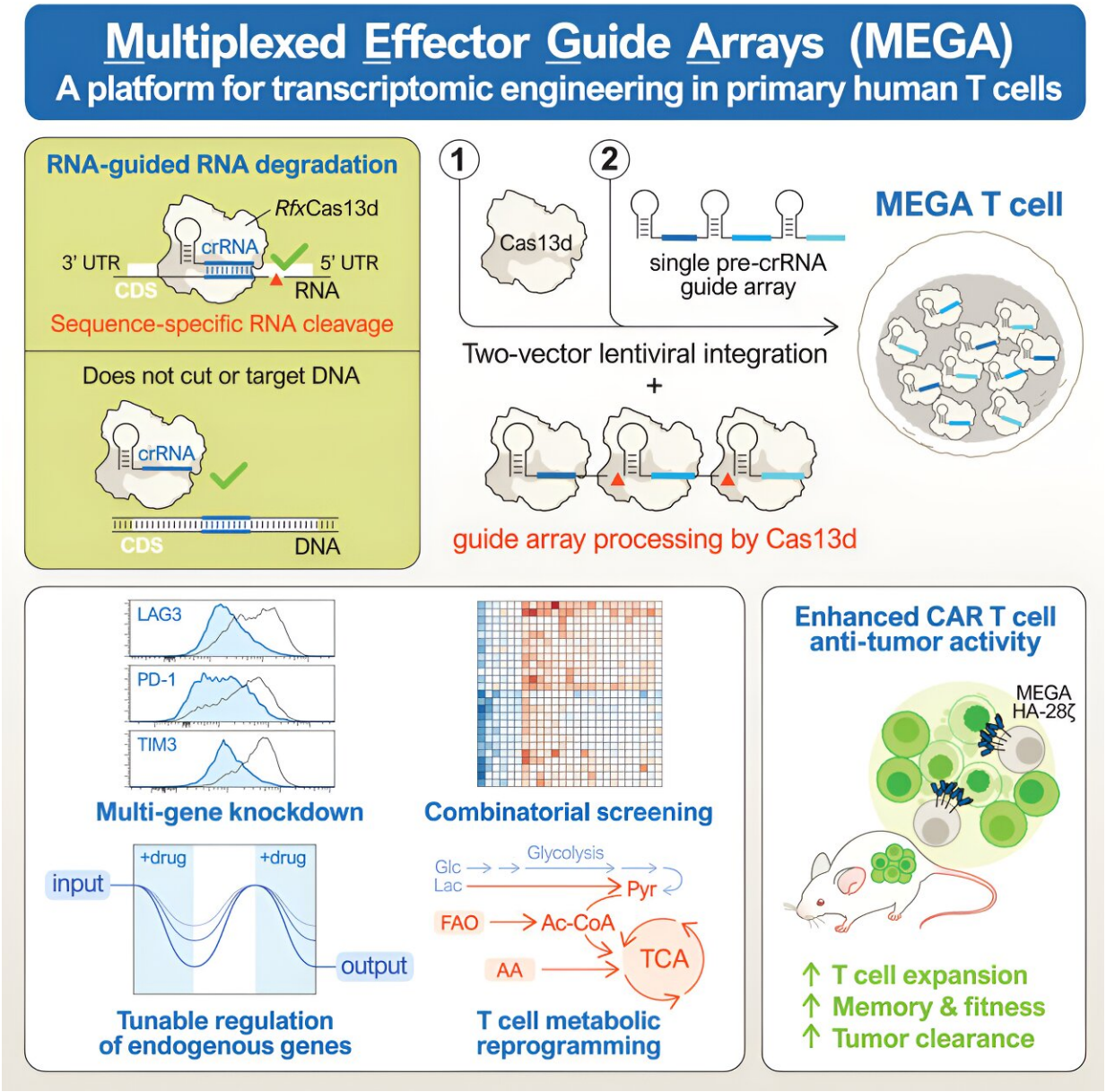

Credit: *Cell* (2024). DOI: 10.1016/j.cell.2024.01.035

Cell therapies for cancer can be potentially enhanced using a CRISPR RNA-editing platform, according to a new study published Feb. 21 in *Cell*.

The new platform, Multiplexed Effector Guide Arrays, or MEGA, can modify the RNA of cells, which allowed Stanford University researchers to regulate immune cell metabolism in a way that boosted the cells' ability to target tumors.

Lead author and Stanford graduate student Victor Tieu was interested in improving chimeric antigen receptor (CAR) T cell therapy. In this cancer treatment, T cells—a type of white blood cell—are engineered with the CAR protein, a receptor that allows the cells to better track down cancer cells. While CAR T therapy has successfully treated blood cancers, including lymphomas and multiple myeloma, the engineered immune cells haven't stacked up well against solid cancers such as pancreatic and lung cancers.

That's because solid tumors have a bulkier structure for the immune cells to penetrate—the cells grow exhausted before they can make headway in destroying tumors. T cells evolved to fire up quickly and attack viruses, which means they often burn through their energy stores too soon when fighting cancer.

"We were really interested in how we can make those cells better to improve clinical outcomes," said Tieu. "A lot of the tools that we have right now just aren't that good."

The researchers tested their tool on CAR T cells in lab cultures with

tumor cells and in mice with cancer. "Our finding is that it performs 10 times better, in terms of reducing the tumor growth and in terms of sustaining long term T cell proliferation," said senior author Stanley Qi, Stanford associate professor of bioengineering and institute scholar at Sarafan ChEM-H.

Stopping cell exhaustion

Previous research efforts to improve CAR T cell therapy have used CRISPR-Cas9 to edit the cells' DNA. However, this gene-editing platform comes with risks because it permanently deletes bits of DNA, which can have unintended consequences and even cause the T cells themselves to turn cancerous.

So the Stanford team pursued a different route, exploring whether CRISPR-Cas13d—which uses a molecular scissor that cuts RNA, not DNA—could enable reversible changes to gene expression in T cells. Unlike Cas9, Cas13d can easily target multiple genes at the same time—in the paper, the researchers demonstrated they could make 10 edits at once to human T cells.

"RNA is the next layer up from DNA, so we're not actually touching any of the genetic code," said Tieu. "But we're still able to get big changes in gene expression that are able to change the behavior of the cell."

To see whether this tool could successfully improve CAR T cell function, they identified 24 genes that could be involved in the T cell exhaustion. They then tested 6,400 paired gene combinations in culture, with different genes turned down using the MEGA tool, and identified new gene pairings that worked especially well together to boost anti-tumor function.

Turning T cells into marathon runners

In another experiment, the team tuned a set of metabolic genes in the T cells to tilt the cells from sprinters to marathon runners, giving them the endurance to chip away at tumors. They compared these MEGA CAR T cells to non-engineered T cells and CAR T cells, both in lab cultures with tumor cells and in mice with cancer. After three weeks, they tested the extent of the tumors as well as how the T cells were surviving.

At first, the MEGA cells lagged in their anti-cancer activity. "Initially, I was like, 'Oh, these cells are worse,'" said Tieu. But, after some time, these cells persevered against the tumor cells while the CAR T and regular T cells wore themselves out, leading to the 10-fold improvement in tumor growth reduction and T cell proliferation.

The secret was shifting how the cells spent their sugar, away from a fast-burning glycolysis process toward favoring oxidative phosphorylation.

"We were able to use this technology to engineer the mRNAs in this sugar-usage pathway inside the T cells that regulate their choice of which sugar molecule to use," said Qi. As a result, "We were able to really sustain the persistence of these T cells, so the T cell could live longer in the tumor site, and also exert much better performance."

Not only did the MEGA platform allow for fine-tuning genes regulating T cell metabolism, the tune-up could also be regulated with a drug. When an antibiotic called trimethoprim was present, it turned on the RNA changes, tamping down on the cells' glycolysis metabolism and turning them into endurance athletes in their attack on the tumor cells. When the drug was gone, the cells reverted to their original gene expression.

This drug-based control mechanism "allows you to create a safety

switch" for immunotherapy treatments, said co-author Crystal Mackall, the Ernest and Amelia Gallo Family Professor and a professor of pediatrics and of medicine at Stanford.

While the platform is still in its early stages, the researchers hope that it can eventually prove useful in clinical settings.

Tieu plans to continue development of the platform toward this goal. "It would be really cool to try to push this to an actual clinical product," he said. "I think there's a lot of potential to really improve CAR T cell therapy in ways that people couldn't have done before."

More information: Victor Tieu et al, A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells, *Cell* (2024). DOI: 10.1016/j.cell.2024.01.035

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