Tweaked CRISPR in neurons gives scientists new power to probe brain diseases

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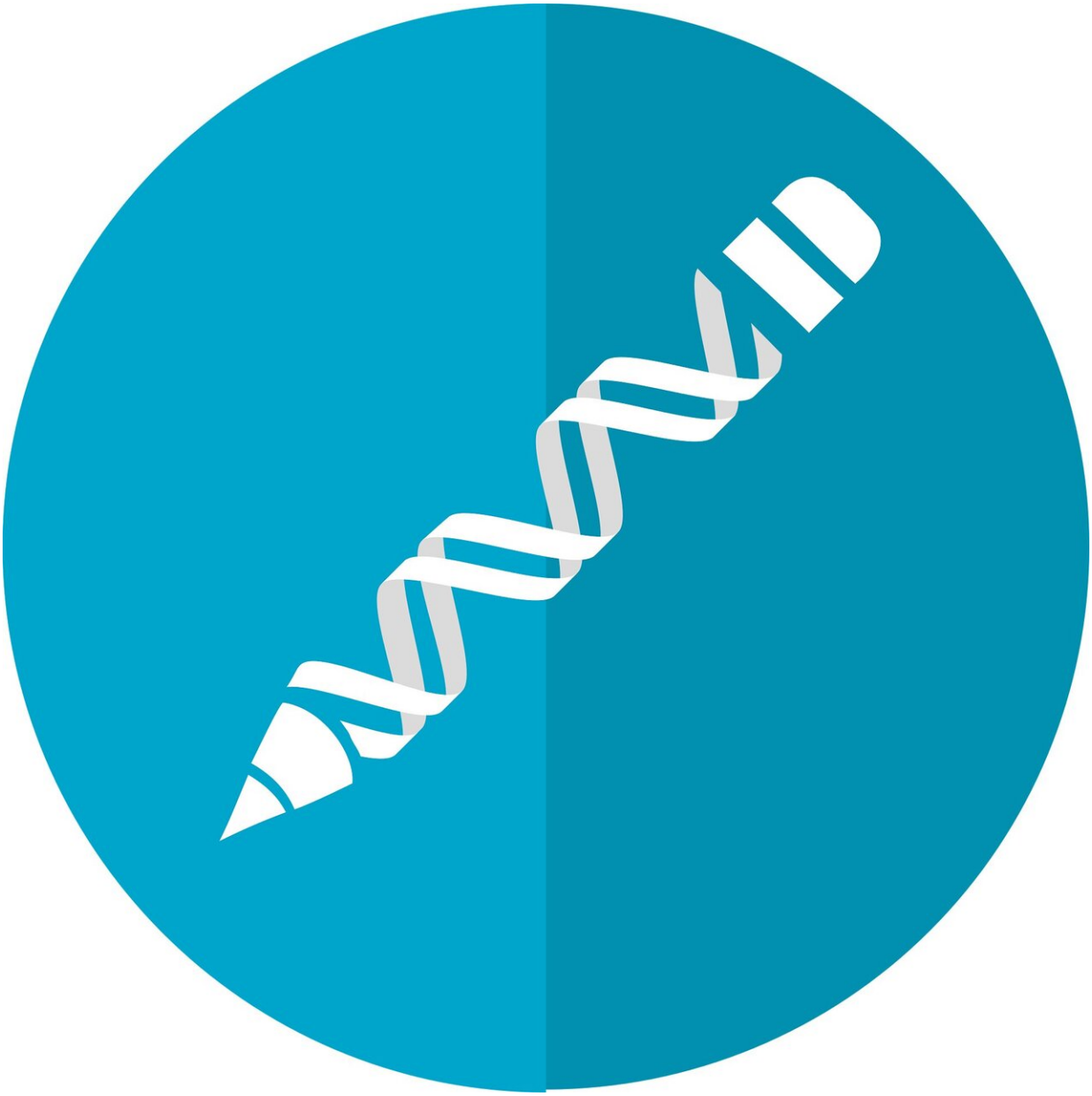

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A team of scientists at UC San Francisco and the National Institutes of Health have achieved another CRISPR first, one which may fundamentally alter the way scientists study brain diseases.

In a paper published August 15 in the journal *Neuron*, the researchers describe a technique that uses a special version of CRISPR developed at UCSF to systematically alter the activity of genes in human neurons generated from stem cells, the first successful merger of stem cell-derived cell types and CRISPR screening technologies.

Though mutations and other genetic variants are known to be associated with an increased risk for many neurological diseases, technological bottlenecks have thwarted the efforts of scientists working to understand exactly how these genes cause disease.

"Prior to this study, there were significant limitations that restricted what scientists could do with human neurons in the lab," said Martin Kampmann, Ph.D., associate professor in UCSF's Institute for Neurodegenerative Diseases, a CZ Biohub Investigator, and co-senior author of the new study.

For one thing, until fairly recently, there was no way for scientists to reliably obtain human brain cells that could be used in advanced lab experiments, explained Kampmann, also a member of the UCSF Weill Institute for Neurosciences. "It was possible to get neurons donated by patients who had undergone procedures that involve removing brain tissue to treat epilepsy or brain cancer. But these samples can only survive for a few days. You can't perform experiments to probe gene function on short-lived neurons."

Instead, scientists have generally relied on animal models of brain disease, which can fail to capture many nuances of human neurobiology.

A breakthrough came in 2006 when Shinya Yamanaka, MD, Ph.D., of Kyoto University and the UCSF-affiliated Gladstone Institutes, discovered a way to rewind the developmental clock and turn adult cells into stem cells that could, with some coaxing, be transformed into any type of cell found in the body—including neurons. These "induced pluripotent stem cells" (iPSCs) made human brain cells widely available for lab research.

When the CRISPR gene-editing system arrived six years later, scientists thought they finally had all the tools they would need to manipulate genes in human neurons and determine how they contribute to neurological disease.

But scientists quickly discovered that the DNA-cutting machinery of the CRISPR system, an enzyme known as Cas9, didn't mix well with iPSCs. "Stem cells have a very active DNA damage response. When Cas9 produces even just one or two DNA cuts, it can lead to toxicity that causes the cells to die," Kampmann said.

So Kampmann decided to tackle the toxicity problem. As a postdoc in the lab of UCSF Professor Jonathan Weissman, Ph.D., Kampmann co-invented a tool known as CRISPRi (for "interference"), a modified form of CRISPR technology in which the Cas9 enzyme has been deactivated. When CRISPRi finds the gene it's seeking, it suppresses its activity without making any cuts. As a result, unlike standard CRISPR-Cas9, Kampmann predicted, CRISPRi shouldn't be toxic to iPSCs or stem cell-derived neurons.

In the new paper, Kampmann and his collaborators describe how they adapted CRISPRi for use in human iPSCs and iPSC-derived neurons,

and found that it could target and interfere with genes without killing the cell—a feat that had long eluded scientists.

Using this system, the researchers demonstrated how their technique can be used to find genes that may cause or contribute to brain diseases. For example, they identified genes that specifically extend the lifespan of neurons, but have no comparable effect on iPSCs or cancer cells. They also found genes that increased the number of neurites—projections that grow from neurons and transmit nerve signals—and determined how frequently they branched.

But one of the most surprising findings was the discovery that "housekeeping" genes—known to be essential for survival, but thought to perform the same function in all cells—actually behave differently in neurons and stem cells. When the researchers interfered with the same housekeeping genes in these two cell types, the cells responded by activating (or inactivating) a vastly different set of genes. This result suggests that, contrary to received wisdom, housekeeping genes may not work the same way in different cell types, an idea that Kampmann and his lab are eager to explore further, as these differences may play important roles in disease.

Kampmann is now using the technology to study different types of neurons in an effort to determine why certain diseases selectively affect just a subset of neurons, such as the way motor neurons are selectively damaged in ALS. He's also expanding his investigations into other types of brain cells—including cells known as astrocytes and microglia—which scientists only recently figured out how to produce from human iPSCs.

But ultimately, the goal is to turn this technology that combines CRISPRi and iPSCs into a tool that uncovers much-needed new therapeutic approaches to treating brain diseases.

"One of the big challenges facing the field is that, for most of these disorders, the precise molecular pathways that we should target for drug development remain unclear," said Michael Ward, MD, Ph.D., co-senior author of the new study and a physician-scientist at the National Institutes of Health.

"With this technology, we can take skin or blood cells from a patient with a neurodegenerative disease like Alzheimer's, turn them into neurons or other brain cells, and figure out which genes control the cellular defects associated with the disease," said Kampmann. "The information may allow us to identify effective therapeutic targets."

More information: *Neuron* (2019). www.cell.com/neuron/fulltext/S0896-6273(19)30640-3

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