Researcher discusses CRISPR-Cas3 as a DNA shredder for gene therapy

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Over the past decade, CRISPR-Cas9 gene editing has revolutionized science. It has been lauded as a breakthrough in biogenetics and medicine, with the potential to treat or eliminate many chronic or genetic diseases.

The technique cuts DNA at a precise target location. Where the cut will

be made is determined by a strand of RNA, called the RNA guide, that bears a sequence of nucleotides complementary to the DNA sequence being targeted. The RNA guide runs through DNA until it locates the exact target sequence. Bound to the RNA guide is the enzyme Cas9, which is often likened to a pair of scissors. Once the RNA guide finds the target sequence, Cas9 cleaves the DNA molecule exactly where the RNA guide indicates. This process allows scientists to inactivate or modify specific genes with greater precision and ease than ever before.

The CRISPR mechanism evolved naturally, probably more than a billion years ago. Scientists have harnessed it for gene editing, but they didn't create it. "The general public may think that CRISPR was born for genome-editing work, but it's actually the workhorse of a natural immunity system found in bacteria and archaea," explains Ailong Ke, Molecular Biology and Genetics. "It fights off viruses by slicing and shredding the viruses' genome into pieces."

As a biochemist with a special interest in RNA biology, Ke has found the CRISPR-Cas system irresistible. He and his lab are dedicated to exploring the mechanisms that underpin it. Using cutting-edge tools such as cryo-electron microscopy and X-ray crystallography—both of which can reveal macromolecular structures at the atomic level—the researchers peer into the gears of the CRISPR-Cas machinery.

"Our fundamental drive is scientific curiosity," Ke says. "We want to understand how the system works. A good understanding of the system leads to interesting ideas about how to utilize it. It's such a powerful tool: It is highly specific. It permanently changes the genetic information inside cells, which has profound consequences. The technology carries great promise to cure rare genetic diseases, or to fight off cancer and viruses."

The power of CRISPR-Cas3

The naturally occurring CRISPR-Cas system in bacteria can be based on any one of a number of enzymes in the Cas family. While Cas9 has been getting all of the press coverage, the Ke lab has been exploring its more complicated cousin, CRISPR-Cas3, which is very popular among bacteria. Almost 50% of the known CRISPR-Cas systems in nature use Cas3, Ke explains.

"Cas3 has different composition and activity than Cas9," he says. "Not only does Cas3 cleave the DNA, but it also shreds it into pieces. The consequences are dramatic. The ability of Cas3 to erase the viral genome completely may have contributed to its popularity among bacteria."

Although it is powerful, CRISPR-Cas3 is rarely used in genome-editing applications because of its sophistication. "It's hard to tame for genome-editing applications in human cells," Ke says. "That's why we started with doing the basic science to first understand how it works in test tubes."

Once Ke and his lab had a good handle on how the system worked, they established collaborations with other researchers—including genome-editing expert Yan Zhang at the University of Michigan—to utilize CRISPR-Cas3 for genome editing in human cells. This work paved the way for CRISPR-Cas3's potential application to treat human genetic diseases and to eradicate viruses, such as herpes and hepatitis B, that infect human cells persistently.

"There are still lots of hurdles we'll need to overcome," Ke says. "For example, how do we deliver our CRISPR-Cas3 tool into cells? We also need to make sure that any CRISPR-Cas3–based therapeutic is both effective and safe."

Game-changing applications

Ke and his lab are looking at multiple applications for their CRISPR-Cas3 tool. "One idea we had was to program it to shred a viral genome into pieces, in the same way a bacteria would use it," Ke says. "But we have another idea that gets us really excited: the possibility of using it as an anticancer treatment."

Ke points out that humanity is entering an era of personalized medicine, wherein therapies will be tailored precisely for individuals. "We will be using ever more powerful therapeutic tools, and we're going to target diseases with ever higher accuracy," he says. "Our CRISPR-Cas3 anticancer application has the potential to be part of that. We're targeting specific mutations in cancer cells to achieve a cure. If our approach turns out to be successful, then this will be really impactful research."

When Ke started out as a scientist in 2008, CRISPR was a new frontier in RNA biology. "There were only about 30 papers published on CRISPR, and I read every one of them," he says. "Today there are tens of thousands of papers published. I can't keep up with them. When a field is healthy like this, you see the publications go up in an exponential curve.

"I remember reading those early papers and wondering what mechanisms led to the behavior of those macromolecules," he continues. "We were all scratching our heads, trying to come up with explanations. When we finally began to understand the mechanisms, the payoff went beyond our wildest dreams."

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