A new CRISPR-Cas9 protein to increase precision of gene editing

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The newly developed SaCas9-HF can be applied in gene editing which requires high precision. Credit: City University of Hong Kong

A team of researchers from City University of Hong Kong (CityU) and Karolinska Institutet has recently developed a new protein that can help

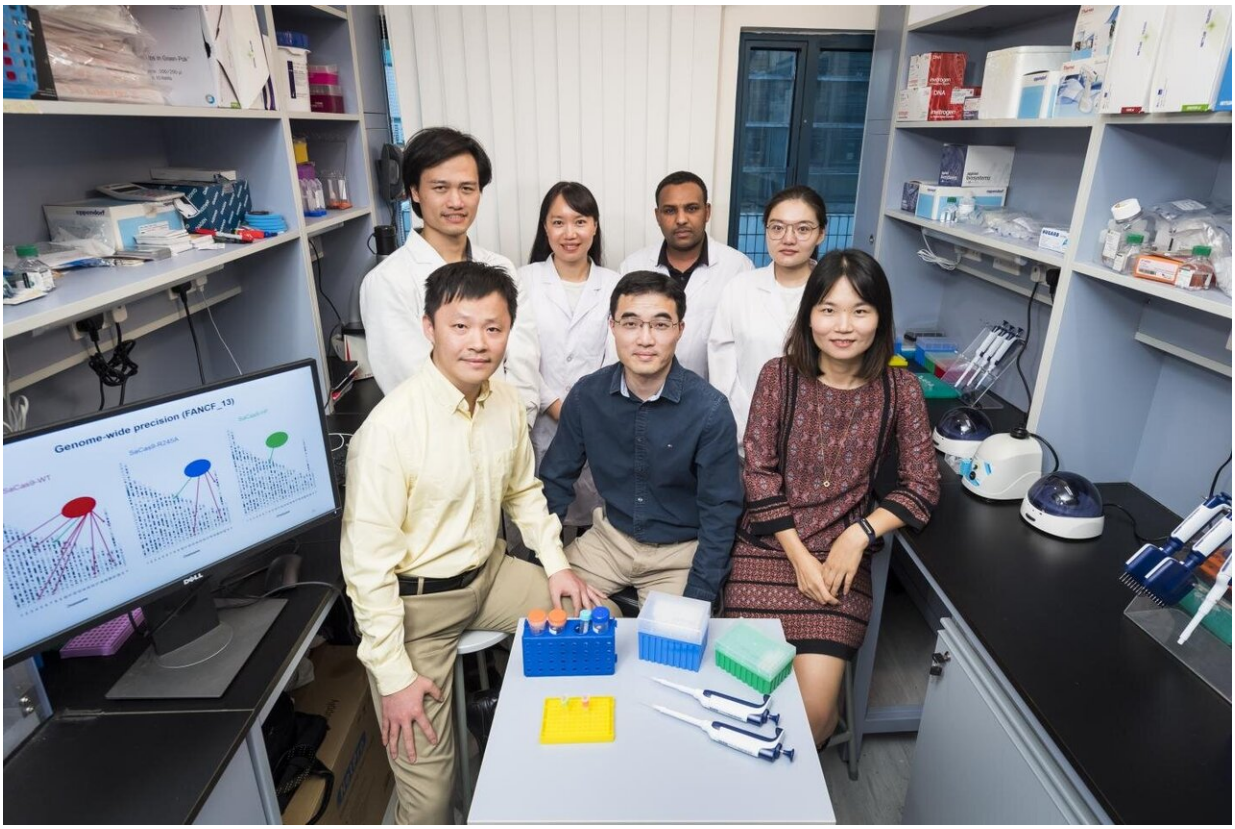
increase the targeting accuracy in the genome editing process. It is believed that it would be useful for future gene therapies in humans, which require high precision.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) -Cas9 is a promising gene-editing technology which could have wide applications, from curing many genetic diseases to developing drought-tolerant crops. Clinical trials using CRISPR-Cas9 to treat cancers, blood disorders, and eye diseases are underway.

Repairing the genetic defects on site

CRISPR-Cas9 is regarded as a powerful tool in gene editing because it has made gene modification or editing very simple. Unlike traditional gene therapy where additional copies of the normal gene are introduced into cells, CRISPR-Cas9 "repairs" the defects on site by removing the problematic DNA or correcting it to restore normal gene functions.

During the process, the Cas9 enzyme is responsible for locating the problematic DNA throughout the genome before making modifications. But it has been found that sometimes it may be not precise enough, and modifications of DNA at unintended places in the genome may happen. Unintended modifications of the genomes could potentially lead to serious consequences, such as cancers, as happened in the initial gene therapy trials years ago. Thus it is important for CRISPR-Cas9 do the "molecular surgery" on the genome precisely.



Team members from CityU include (from left to right, front row) Dr Shi Jiahai, Dr Zheng Zongli, Dr Xiong Wenjun, (from left to right, back row) Hoang Anh Duc, Tan Yuanyan, Firaol Tamiru Kebede and Bao Siyu. Credit: City University of Hong Kong

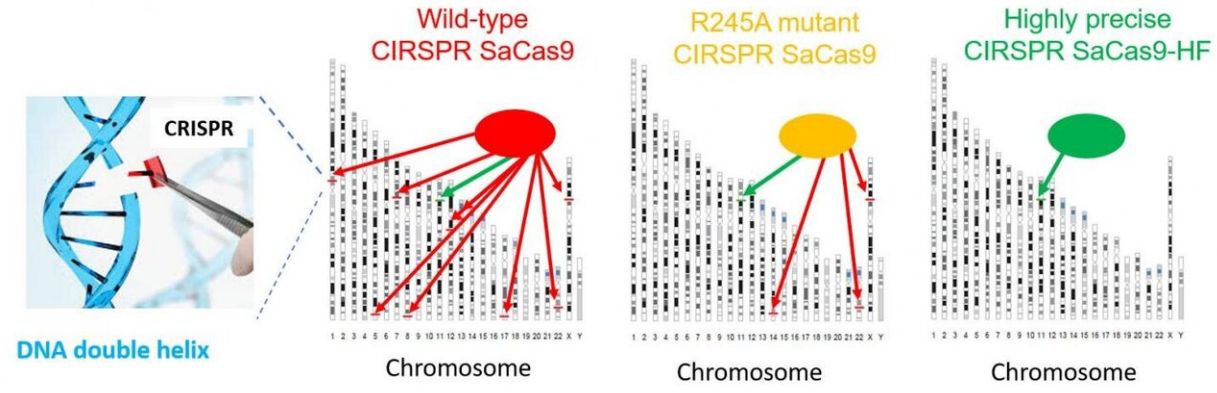
Currently, there are two versions of Cas9, namely SpCas9 (meaning Cas9 nuclease from the bacteria *Streptococcus pyogenes*) and SaCas9 (Cas9 nuclease from *Staphylococcus aureus*), which are commonly used in CRISPR. Both of them have a certain level of imprecision or off-target effect. Researchers have already engineered SpCas9 variants, meaning modified SpCas9s, to improve SpCas9's targeting precision. But these can be too large to fit in the small delivery vector named adeno-associated viral (AAV) vector that is commonly used for in vivo gene therapy.

On the contrary, SaCas9 is much smaller than SpCas9 and can be easily packaged in the payload-limited AAV vectors for delivering gene-editing components in vivo. However, no SaCas9 variant with high genome-wide targeting accuracy is available.

SaCas9-HF dramatically improved genome-wide targeting accuracy

In a recent research led by Dr. Zheng Zongli, Assistant Professor of Department of Biomedical Sciences at CityU and the Ming Wai Lau Centre for Reparative Medicine of Karolinska Institutet in Hong Kong, and Dr. Shi Jiahai, Assistant Professor of Department of Biomedical Sciences at CityU, the team has successfully engineered SaCas9-HF, a CRISPR Cas9 variant which has high accuracy in genome-wide targeting in human cells without compromising on-target efficiency.

The research team's finding was based on a rigorous evaluation of 24 targeted human genetic locations comparing the original unmodified (wild-type) SaCas9 and the new SaCas9-HF. For those targets having highly similar sequences in the genome and hence prone to off-target editing by the wild-type enzyme, SaCas9-HF reduced the off-target activity by about 90%. For many of those targets with relatively less off-target editing by the wild-type enzyme, SaCas9-HF yielded almost no detectable off-target activity.



Comparison of the precision with different types of SaCas9: the green arrows shows the target of gene editing, while the red arrows are the off-targets. The new SaCas9-HF has the highest accuracy. Credit: Dr Zheng Zongli

An alternative to SaCas9 genome-editing applications

"Our development of this new SaCas9 provides an alternative to the wild-type Cas9 toolbox, where highly precise genome editing is needed. It will be particularly useful for future gene therapy using AAV vectors to deliver a genome editing 'drug' in vivo and would be compatible with the latest 'prime editing' CRISPR platform, which can 'search-and-replace' the targeted genes," said Dr. Zheng.

The research findings were published in the scientific journal *Proceedings of the National Academy of Sciences (PNAS)* titled "Rationally engineered *Staphylococcus aureus* Cas9 nucleases with high genome-wide specificity."

More information: Yuanyan Tan et al, Rationally engineered Staphylococcus aureus Cas9 nucleases with high genome-wide specificity, *Proceedings of the National Academy of Sciences* (2019). DOI: 10.1073/pnas.1906843116

Provided by City University of Hong Kong

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