**[A Review about a New Technique in Genome Editing]**

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**Abstract:**

**Similar to the RNA-guided endonuclease Cas9, the Argonaute protein family can also use oligonucleoti-des as guides to degrade invasive genomes. Recently, some Chinese researchers have developed a new technique for genome editing in human cells, which uses a DNA-guided endonuclease named *Natronobacterium gregoryi* Argonaute(NgAgo). Compared to CRISPR-Cas9, this technique has many differences and new features. Although this technique is not as mature as CRISPR-Cas9, it has a good prospect according to the research.**

**Introduction**

CRISPR-Cas9 is a genome editing technique widely used in gene engineering. It guides DNA-RNA hybridization to cut DNA sequence specifically.It has some disadvantages, such as needing PAM(protospacer-adjacent motif) , guide-target mismatches, interference from endogenous RNA,etc.As stated in [1], although there are many modifications to improve its efficiency and specificity,its practical utility is still limited.

Argonautes are a family of endonucleases that use 5’phosphorylated short single-stranded(ss) nucleic acids as guides to cleave targets. Similar with Cas9, Argonautes play important roles in gene expression repression and defense against foreign nucleic acids by cleaving them, so there possibly exists a way to use Argonautes to edit gene like Cas9 does.

**Recent Progress**

Chinese researchers have discovered a kind of Argonaute , *Natronobacterium gregoryi,* (NgAgo)which can be programmed with single-stranded DNA guides at 37℃, and is a precise and efficient tool for genome editing in mammalian cells[1].

**Discussion**

Argonautes are known in RNA-silencing process in eucaryon.Although most Argonautes associate with ssRNA and participate in RNA silencing, some of them can bind ssDNA and cleave the target DNA.Cas9 only exists in prokaryotes, when Argonautes exist in almost all species. For correct Cas9 binding, there must be a 3’ RNA-RNA hybridization structure in the guide RNA, but no specific secondary structure of guides is required for Argonaute binding. Cas9 needs PAM to locate the target when Argonautes do not need it.

From the compare between the Cas9 and Argonautes, we know that Argonautes can do better in some respects. According to some research[2], Argonautes are extract from thermophiles which live in a temperature higher than 60℃, so Argonautes usually works at a temperature between 60℃ to 100℃, at which DNA may be unstable.so the discovery of NgAgo enable us to cleave DNA by Argonautes in a room temperature.

As for evaluating a genome editing tech-nique , we care about these properties: a) how many target sequences it support; b) efficiency;

c)convenience; d)the length of sequence it can recognize; e)the rate of missing target sequence; f)whether it is expandable or not (modulari-zation).

No matter Cas9, Cpf1 or CRISPR-cascade, they all need PAM to recognize the target sequence. PAM limited their supporting range. NgAgo does not need PAM , so it can work anywhere theoretically. Experiments confirmed that NgAgo-gDNA system has a high efficiency in editing mammalian genome[1].NgAgo use ssDNA, which is much more convenient than complicated procedures before CRISPR-Cas9. NgAgo recognize sequence in length of 23~25nt, while CRISPR-Cas9 recognize 19nt.Researchers tested the tolerance of mismatch, and the results show that the efficiency decrease obviously when one base pair mismatched ,and when more than one base pair mismatched ,NgAgo almost stop working.Thees results could be explained that DNA-DNA bind is more accurate than DNA-RNA bind.

NgAgo do not need any special structures , which causes some new problems.As for human, there is not any ssDNA in our cells.But there are lots of species whose cells have too much endogenous ssDNA to use NgAgo. NgAgo do not need any special structures so that it can bind any ssDNA in cells. So if cell has a high content of ssDNA, NgAgo may not be suitable for its genome editing.Another problem is about delivering. If cell contains little ssDNA(so that we can use NgAgo), we must deliver ssDNA into the cell because cell could not produce ssDNA itself.

When we do editing rather than deletion, we need to import a new homologous ssDNA as a template.This ssDNA must be long enough , otherwise it will make gene orthogonality problems.

The limitation of length makes AgNgo has almost nothing to do with expandability.Its 25nt basic groups are all used to recognize, so it is difficult to design other functions on ssDNA besides genome editing.

NgAgo enable a new method about genome editing.As a technique, CRISPR-Cas9 has improved our research to a large extent. What will NgAgo bring to us ? Let us wait and see.

**References**

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