A.s. Cas12a, a highly effective CRISPR genome editing enzyme

Zhang et al. (*Nat Comms.* 2021;12:3908) demonstrate high potency and specificity of Cas12a *Ultra*

Citation summary: Starting with the sequence of *Acidaminococcus sp.* Cas12a, Zhang et al. [1] developed a new protein, "AsCas12a *Ultra*," with increased genome editing potency and precision. In a variety of cell lines, this CRISPR nuclease demonstrated nearly 100% editing efficiency at all genomic sites tested. In T cells, greater than 90% efficiency was observed when three genomic sites were targeted simultaneously. Knocking in transgenes also demonstrated up to a 60% success rate.

Background

Cas9 genome editing, while valuable for many research purposes, is restricted to genomic sites containing a Cas9- recognizable protospacer-adjacent motif (PAM). GC-rich regions are more amenable to Cas9 genome editing than are AT-rich regions. Cas12a recognizes TTTV PAM sequences and thus is frequently the CRISPR-Cas endonuclease of choice for editing in AT-rich regions. Wild-type (WT) Cas12a also compares favorably to WT Cas9 in terms of site specificity. However, WT Cas12a has been shown to have significantly less potency than wild-type Cas9. Zhang et al. developed AsCas12a *Ultra*, a modification of *A.s.* Cas12a that would maintain site specificity while demonstrating high levels of editing activity.

Experiment and results

As described in a previous **DECODED article, a process of selecting and enriching for A.s. Cas12a mutations with increased cleavage efficiency** was performed in *E. coli*. Multiple rounds of selection and enrichment for higher-activity Cas12a mutants resulted in the isolation of M537R/F870L (AsCas12a *Ultra*). Then, using **Spec/SEAM-seq**, the researchers found that AsCas12a *Ultra* had binding and cleavage specificities similar to WT Cas12a. As described in the aforementioned DECODED article, AsCas12a *Ultra* also showed **greatly improved potency** over WT Cas12a, including in low temperature conditions (30°C).

Using a variety of primary cell types (T cells, HSCs, NK cells, and iPSCs), the researchers employed the electroporation of ribonucleoproteins (RNPs) consisting of CRISPR RNAs (crRNAs) combined with either WT or AsCas12a *Ultra* to edit many different genomic target loci. Concentration-response curves demonstrated an increase in editing efficiency with AsCas12a *Ultra*. Editing efficiencies of WT and AsCas12a *Ultra* were investigated by next generation sequencing (NGS) and demonstrated to be equivalent.

The researchers investigated an approach to develop allogenic T cells by triple knockout of TRAC, B2M, and CIITA using AsCas12a *Ultra*. Greater than 90% editing efficiency was observed at all three genomic sites. In a follow-up experiment, a donor template carried by AAV6 was introduced to allow expression of a transgene. Genes for green fluorescent protein (GFP) and mCherry were successfully introduced at close to 60% knock in rates—even double knock-in of these two fluorescent reporters was achieved at a greater than 20% rate.

Finally, the researchers studied the ability of AsCas12a *Ultra* to generate allogenic chimeric antigen receptor (CAR) NK cells. First, they used AsCas12a *Ultra* in NK cells to knock out the TGFBR2 gene, which codes for one subunit of the TGF-β receptor. They showed that in response to TGF-β, the edited NK cells more effectively killed SK-OV-3 ovarian tumor spheroids than unedited NK cells while also exhibiting lower levels of SMAD2/3 phosphorylation, explaining the mechanism of this cytotoxic effect. Then, using AAV6, the researchers knocked in a CAR which targets the epidermal growth factor receptor (EGFR). The resultant knock-in cells (αEGFR-CAR+ NK) were then co-cultured with EGFR+ PC-3 prostate tumor spheroids, which lead to decreased spheroid size. The data demonstrated that the cytotoxicity of NK cells was increased by these genome editing modifications.

Conclusion

The researchers concluded that AsCas12a *Ultra* is capable of being used in research to edit and knock in transgenes that enhance the function of effector cells. They also mentioned that the crRNA, needed for use with this enzyme, is shorter than the sgRNA used for Cas9 endonucleases, thus decreasing research costs, especially in the case of whole-genome screening analyses. The efficiency and specificity of AsCas12a *Ultra* makes this a top choice for genome editing research. This protein is available for research use only as **Alt-R™ A.s. Cas12a (Cpf1) *Ultra*** from IDT. These research data demonstrate the advantages of Alt-R A.s. Cas12a (Cpf1) *Ultra*, an engineered AsCas12a nuclease variant, as a tool to eventually enable the development of gene-edited cell medicines.

References

Zhang L, Zuris JA, Viswanathan R, et al. *Nat Comms. 2021;12(1):3908*.

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