GENETICS AND GENODERMATOSES

TYPE I CRISPR-CAS3 INDUCES BROARD, DISTAL AND UNIDIRECTIONAL GENOME EDITING IN HUMAN CELLS

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Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated (Cas) is an adaptive immune system in prokaryotes. The CRISPR-Cas systems are taxonomically grouped as Class 1 and Class 2.
Although single-component Class 2 CRISPR systems, such as type II Cas9, are widely used for genome editing in eukaryotic cells, the application of multi-component Class 1 CRISPR has yet to be realized. The Class 1/Type I CRISPR system functions as a CRISPR RNA (crRNA)-bound multiprotein complex, termed CRISPR-associated complex for antiviral defence (Cascade), and a Cas3 endonuclease, which is recruited upon target binding by Cascade to cleave foreign DNA.
Here, we demonstrate that type I-E CRISPR, composed of Escherichia coli Cascade, Cas3, and programmable pre-crRNA, mediates distinct DNA cleavage activity in human cells. Notably, Cas3, which possesses helicase and nuclease activity, predominantly triggered several thousand base pair deletions upstream of the 5'-ARG protospacer adjacent motif (PAM). Whole genome sequencing and capture-based deep sequencing indicated that CRISPR-Cas3 has comparable or even lower off-target activity compared with CRISPR-Cas9.
A surrogate reporter assay demonstrated CRISPR-Cas3 was more appropriate than CRISPR-Cas9 for “remote” genome editing including knock-outs and knock-ins. We sought to use the CRISPR-Cas3 system for therapeutically relevant genome editing by inducing exon skipping in the dystrophin (DMD) gene. We used a Firefly luciferase-based exon

skipping reporter model of human DMD. CRISPR-Cas3 showed significantly higher levels of exon skipping than CRISPR-Cas9 with the two sgRNAs.
Large deletions of mutated allele would be introduced by CRISPR-Cas3, so that it induces exon skipping of dominant negative mutations to attenuate skin diseases with autosomal dominant inheritance, such as autosomal dominant epidermolysis bullosa. These findings broaden our understanding of the Class 1 CRISPR system, which may serve as a novel and unique genome editing tool in eukaryotic cells in a manner distinct from the Class 2 CRISPR system.