CRISPR/Cas9 inhibits rather than induces non-targeted DNA cleavage more likely to cause off-target single-nucleotide variants

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September 25, 2021

Abstract

CRISPR/Cas9 gene targeting technology has become the most widely used gene editing technology in both plants and animals. However, substantial off-target effect remains as a major imperfection hindering its further application. Here, Nicotiana benthamiana leaf cell-free system was used to simulate in vivo environment. And the effects of different CRISPR/Cas9 components on DNA stability in cell-free system were studied to explore possible mechanisms causing CRISPR off-target. The results showed that overexpressing Cas9, nCas9 and dCas9 significantly inhibited DNA cleavage in the cell extracts. While overexpressing RNPs accelerated the target DNA cleavage but inhibited non-target DNA digestion in cell extracts, overexpressing nRNP and dRNP blocked the cleavage of either target or non-target sequences. Meanwhile, analysis of whole-genome sequencing data from mice and rice edited by different CRISPR tools revealed that the main off-target mutations were SNVs (single nucleotide variants), rather than Indels (insertions and deletions) that were readily induced by DNA double-strand breaks. The off-target sites did not match the conventionally predicted places but were PAM-rich sites preferred. Our study suggests that PAM-dependent binding without cleavage of CRISPR/Cas9 to non-target sequences may increase off-target mutation risks through impeding the necessary cleavage process for repairing spontaneous or environmentally induced non-targeted DNA mutations.

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