МОЛЕКУЛЯРНАЯ БИОЛОГИЯ, 2024, том 58, № 1, с. 157–159

МОЛЕКУЛЯРНАЯ БИОЛОГИЯ КЛЕТКИ

УДК 577.151:575.2

РАЗРАБОТКА ВЫСОКОСПЕЦИФИЧНЫХ И ЭФФЕКТИВНЫХ ВАРИАНТОВ ЭНДОНУКЛЕАЗЫ SpCas9 НА ОСНОВЕ HH-ТЕОРИИ[#]

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Поступила в редакцию 01.04.2023 г.
После доработки 31.07.2023 г.
Принята к публикации 15.08.2023 г.

Эндонуклеаза Cas9 *Streptococcus pyogenes* (SpCas9) – самый популярный инструмент редактирования генов, но нецелевой мутагенез, сопровождающий ее действие, представляет серьезное ограничение. Ранее нами предложена HH-теория, в которой утверждается, что индуцированное экструзией гибрида sgRNA/DNA (hibrid) усиление гидрофобных взаимодействий (hydrophobic interaction) между гибридом и REC3/HNH является ключевым фактором инициации расщепления. Теперь на основе HH-теории проанализировано взаимодействие домена REC3 с гибридом и получено 8 мутантных сайтов. Мы сконструировали 8 вариантов SpCas9 (V1–V8), использовали цифровую капельную ПЦР для оценки SpCas9-индуцированных инделей ДНК в клетках человека и разработали высокоточные варианты эндонуклеазы. Таким образом, HH-теория может быть использована для дальнейшей оптимизации систем редактирования генома, опосредованных SpCas9, а полученные варианты V3, V6, V7 и V8 SpCas9 можно рассматривать как перспективный инструмент для приложений, требующих высокоточного редактирования генома.

Ключевые слова: SpCas9, HH-теория, варианты, внецелевое расщепление, эффективность расщепления, оптимизация

DOI: 10.31857/S0026898424010158**, EDN:** NTCLJI

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[#]Полный текст статьи на английском языке размещен на сайте издательства Springer – https://link.springer.com/journal/11008

[§]Авторы внесли равный вклад.

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The Development of SpCas9 Variants with High Specificity and Efficiency Based on the HH Theory[§]

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Streptococcus pyogenes Cas9 (SpCas9) is the most popular tool in gene editing; however, off-target mutagenesis is one of the biggest impediments in its application. In our previous study, we proposed the HH theory, which states that sgRNA/DNA hybrid (hybrid) extrusion-induced enhancement of hydrophobic interactions between the hybrid and REC3/HNH is a key factor in cleavage initiation. Based on the HH theory, we analyzed the interactions between the REC3 domain and hybrid and obtained 8 mutant sites. We designed 8 SpCas9 variants (V1–V8), used digital droplet PCR to assess SpCas9-induced DNA indels in human cells, and developed high-fidelity variants. Thus, the HH theory may be employed to further optimize SpCas9-mediated genome editing systems, and the resultant V3, V6, V7, and V8 SpCas9 variants may be valuable for applications requiring high-precision genome editing.

Keywords: SpCas9, HH theory, variants, off-target, cleavage efficiency, optimization