Genes & Diseases (2014) 1, 6–7

HOSTED BY

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

EDITORIAL

CRISPR clear? Dimeric Cas9-Fok1 nucleases improve genome-editing specificity

KEYWORDS
Cas9;
CRISPR;
Genome editing;
sgRNA;
TALENs;
ZFNs

Bacteria and archaea have been known for decades having evolved adaptive immune defenses called clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems to degrade foreign nucleic acids. Recently, these RNA-guided Cas9 nucleases derived from CRISPR/Cas systems have shown promise in transforming our ability to edit mammalian genomes.[1] While zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have shown similar promise, the ease of producing targeting RNAs over the generation of unique sequence-directed nucleases to guide site-specific modifications makes the CRISPR/Cas9 system an appealing method for genome editing. A short guide RNA (sgRNA) can direct Cas9 to a specific genomic sequence where it induces double-strand breaks that, when imperfectly repaired, yield mutations.[1] Cas9 can also catalyze gene replacement through homologous recombination.[1] Undoubtedly, Cas9-mediated genome editing and regulation should have transformative potential for basic science, genome engineering and therapeutics.[1]

However, the specificity of CRISPR/Cas9 targeting technology remains problematic. It's been noted that sgRNA-Cas9 complexes are in general tolerant of 1∼3, occasionally more, mismatches in their targets, as Cas9 was shown to bind to many sites in human genome other than the desired one.[2] Thus, imperfect Cas9 specificity is a major concern, while several attempts have been made to improve CRISPR specificity, such as using multiple sgRNA-Cas9 complexes for activity, discovering improved natural Cas orthologs, engineering improved Cas9 variants and judiciously choosing targeting sgRNAs.[3]

Two recent studies reported an innovative approach to addressing the problem of off-target genomic cleavage using CRISPR-Cas9 system.[4,5] Both studies took advantage of the known feature of dimerization dependence for Cas9-mediated cleavage and the proven specificity of ZFNs and TALENs. To achieve this feat, the investigators engineered dimeric nucleases by fusing dCas9 with the Fok1 nuclease, which is the obligatory dimeric nuclease used in ZFNs and TALENs.[4,5] The resulting Cas9-Fok1 fusion constructs contain that Fok1 nuclease is fused to the N-terminus of inactive Cas9 in the opposite orientation to that in ZFN and TALENs. The researchers showed that in the optimal configuration the on-target cleavage efficiency of these dimeric Cas9-Fok1 fusions is slightly lower than or comparable to that of wild-type Cas9.[4,5] The requirement for two gRNAs for precise orientation of the target sites and for correct spacing between half-sites is more stringent for the Cas9-Fok1 fusion construct than that for Cas9 nickase, which is a Cas9 variant that cleaves one DNA strand only. Deep sequencing analysis of the previously identified off-target sites of wild-type Cas9 revealed the dimeric Fok1-dCas9 fusion construct has significantly lower off-target activity than that of wild-type Cas9 nuclease.[4,5] One major caveat of this approach is that the more stringent sequence requirements of the dimeric Fok1-dCas9 nucleases will likely reduce the number of available on-target sites. Nonetheless, the requirement of binding to two precisely disposed genomic targetable half-sites should render Cas9-Fok1 fusion nucleases substantially improved on-target specificity. In a broader sense, the development of dimeric Fok1-dCas9 fusions may prove a significant turning point towards practical mammalian genome manipulations with high efficiency and accuracy.

Peer review under responsibility of Chongqing Medical University.

http://dx.doi.org/10.1016/j.gendis.2014.08.004

Acknowledgments

The authors declare no conflict of interest. Work in the corresponding author's laboratory was supported in part by research grants from the National Institutes of Health (AT004418 and CA106569 to TCH).

References

1. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;157(6):1262—1278.
2. Kuscu C, Arslan S, Singh R, Thorpe J, Adli M. Genome-wide analysis reveals characteristics of off-target sites bound by the Cas9 endonuclease. *Nat Biotechnol*. 2014;32(7):677—683.
3. Cho SW, Kim S, Kim Y, et al. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Res*. 2014;24(1):132—141.
4. Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. *Nat Biotechnol*. 2014;32(6):577—582.
5. Tsai SQ, Wyvekens N, Khayter C, et al. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. *Nat Biotechnol*. 2014;32(6):569—576.

Hongmei Zhang
Zhengjian Yan
Melissa Li
Michael Peabody
Tong-Chuan He*
Molecular Oncology Laboratory,
The University of Chicago Medical Center,
Chicago, IL 60637, USA

*Corresponding author.
E-mail address: tche@bsd.uchicago.edu (T.-C. He)

3 August 2014
Available online 22 August 2014