Enhanced Efficiency of Human Pluripotent Stem Cell Genome Editing through Replacing TALENs with CRISPRs

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Transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems are new classes of genome-editing tools that target desired genomic sites in mammalian cells (Miller et al., 2011; Hockemeyer et al., 2011; Cong et al., 2013; Mali et al., 2013; Jinek et al., 2013). TALENs bind as a pair around a genomic site in which a double-strand break (DSB) is introduced by a dimer of FokI nuclease domains. Recently published type II CRISPR/Cas systems use Cas9 nuclease that is targeted to a genomic site by complexing with a synthetic guide RNA that hybridizes a 20-nucleotide DNA sequence (“protospacer”) beginning with G and immediately preceding an NGG motif recognized by Cas9—constituting a G(N)19NGG spacer—resulting in a DSB. The Cas9 nuclease is recruited to the NGG motif (Jinek et al., 2012). However, it is generated, the DSB is double-stranded and can be repaired by homology-directed repair (HDR), which can be exploited with the use of an exogenously introduced repair template to knock in or correct a mutation in the genome.

We recently reported the use of a TALEN genome-editing system to rapidly and efficiently generate mutant alleles of 15 different genes in human pluripotent stem cells (hPSCs) as a means of performing rigorous disease modeling (Ding et al., 2013); the proportions of clones bearing at least one mutant allele ranged from 2%–34%. Although one example of the use of CRISPRs in hPSCs has been reported (Mali et al., 2013), the efficiency of allele targeting was only 2%–4%, and this study, unlike our approach, did not involve a cell-sorting step.

We sought to compare the relative efficacies of CRISPRs and TALENs targeting the same genomic sites in the same hPSC lines with the use of the same delivery platform as we described previously (Ding et al., 2013). In the TALEN genome-editing system, we used the CAG promoter to cotranslate (via a viral 2A peptide) each TALEN with green fluorescent protein (GFP) or red fluorescent protein (RFP). For CRISPRs, we subcloned a human-codon-optimized Cas9 gene with a C-terminal nuclear localization signal (Mali et al., 2013) into the same CAG expression plasmid with GFP, and we separately expressed the guide RNA (gRNA) from a plasmid with the human U6 polymerase III promoter (Mali et al., 2013). The 20 nucleotide protospacer sequence for each gRNA was engineered to bind on methylated DNA. It is possible that we observe.

CRISPRs readily generated homozygous mutant clones (7%–25% of all clones; Table S1) as discerned by sequencing. We also attempted to knock in E17K mutations into AKT2 using a 67 nucleotide single-stranded DNA oligonucleotide as previously described (Ding et al., 2013). Although the predicted CRISPR cleavage site was 11 and 13 nucleotides from the point mutations, respectively, the CRISPR yielded knockin clones at a rate of 11%, whereas TALENs yielded only 1.6% (Table S1).

We speculate that the superior performance of CRISPRs could include (1) the Cas9 protein being more highly expressed and better tolerated than TALENs in hPSCs, as we routinely observed earlier (<24 hr versus 48 hr), and (2) more robust (5%–10% of cells versus <1%–2% of cells) GFP expression following electroporation. Other factors may include intrinsic DNA-unwinding activity of Cas9 and impaired TALEN binding on methylated DNA. It is possible that further optimization of the TALEN system that we developed could improve its efficiency and reduce the differential that we observe.
Two potential disadvantages of CRISPRs are worth noting. First, the requirement for a G(N)_{19}NGG target sequence somewhat limits site selection. Because either DNA strand can be targeted, a target sequence occurs on average every 32 base pairs. This is no barrier for gene knockout, where any coding sequence can be targeted, but it may present difficulties when trying to knock in or correct a mutation at a specific location. However, the requirement for a G at the start of the protospacer is dictated by the use of the U6 promoter to express the gRNA, and alternative CRISPR/Cas systems can relieve this requirement (Cong et al., 2013).

Second, the extent of CRISPR off-target effects remains to be defined. Previous analyses have suggested that one-nucleotide mismatches in the first half of the protospacer are better tolerated than mismatches in the second half (Jinek et al., 2012; Cong et al., 2013). None of the genomic sequences we targeted with CRISPRs have perfectly matched or one-mismatch sequences elsewhere in the genome. For the AKT2 sequence, there is a two-mismatch sequence differing at nucleotides 1 and 3, in the more “tolerant” half of the protospacer; we obtained zero clones with mutations at this potential off-target site, as compared to 61% at the on-target site (Table S1), suggesting that at least in this instance off-target effects are not likely to be a significant concern. Judicious selection of target sites may well be able to minimize systematic off-target effects. Nevertheless, clear-cut determination of the relative risk for both TALEN- and CRISPR-based approaches will require a systematic analysis.

It is important to highlight that all of these genome-editing technology approaches are still very much in development, and more detailed and comprehensive studies will be needed to determine their relative merits in different experimental circumstances. From a practical standpoint, CRISPRs are easier to implement than TALENs, as each TALEN pair must be constructed de novo, whereas for CRISPRs the Cas9 component is fixed and the gRNA requires only swapping of the 20-nucleotide protospacer. Given this consideration and our observations of substantially increased efficiency through replacing TALENs with CRISPRs in an otherwise identical system, we would suggest that CRISPRs might well prove to be a very powerful and broadly applicable tool for the stem cell community.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes one table and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2013.03.006.

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