Crispr/Cas9-mediated cleavages facilitate homologous recombination during genetic engineering of a large chromosomal region

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Abstract

Homologous recombination over large genomic regions is difficult to achieve due to low efficiencies. Here, we report the successful engineering of a humanized mTert allele, hmTert, in the mouse genome by replacing an 18.1-kb genomic region around the mTert gene with a recombinant fragment of over 45.5-kb, using homologous recombination facilitated by the Crispr/Cas9 technology, in mouse embryonic stem cells (mESCs). In our experiments, with specific sites of DNA double strand breaks (DSBs) by Crispr/Cas9 system, the homologous recombination efficiency was up to 11% and 16% in two mESC lines TC1 and v6.5, respectively. Overall, we obtained a total of 27 mESC clones with heterozygous hmTert/mTert alleles and 3 clones with homozygous hmTert alleles. DSBs induced by Crispr/Cas9 cleavages also caused high rates of genomic DNA deletions and mutations at small guide RNA (sgRNA) target sites. Our results indicated the Crispr/Cas9 system significantly increased the efficiency of homologous recombination-mediated gene editing over a large genomic region in mammal cells, but also inherently caused mutations at unedited target sites. Overall, this strategy provides an efficient and feasible way for manipulating large chromosomal regions.

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