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DEVELOPMENT OF ALL-RNA CRISPR-SYSTEM FOR HIGH-EFFICIENCY GS DELETION IN CHO CELLS

Aim. To establish a high-efficiency MAD7 nuclease system for deletion of glutamine synthetase (GS) in CHO-K1 cells, engineering an optimized host for therapeutic recombinant protein production. **Methods.** GS gene (Glul) targeting crRNAs was designed using Chopchop tool and screened by in vitro assay using MAD7-containing lysates, IVT crRNAs and Glul PCR fragments. CHO-K1 editing was done by electroporation of codon-optimized MAD7 IVT mRNA (30 nM), crRNA was delivered either as an expressing pDNA (30 nM) or synthetic oligo (3 μ M). Single site edit efficiency was assessed by T7 endonuclease I (T7E) mismatch assay. Gene-size deletion was done by co-transfecting MAD7 mRNA together with 2 crRNAs flanking Glul. Single cell clones (SCC), obtained by FACS, were screened by PCR amplifying deleted region junction and control intact region (delPCR) to verify biallelic deletion. For Glul^{-/-} phenotype validation selected clones were compared for growth in media with and without L-glutamine. **Results.** 7 active crRNA candidates identified by in vitro screening were validated in CHO-K1 cells. crRNA 3 and 14 showed the highest edit efficiencies in T7E assay (69% and 63%). This pair flanks the 6kb region of 10kb Glul, spanning coding sequences of exons 2 to 7. CHO-K1 cells were co-transfected with crRNA 3 and 14 expressing pDNA and MAD7 mRNA, and then subjected to SCC. Out of 52 scaled-up clones delPCR identified 7 homozygous and 2 heterozygous deletions, representing a 15.6% alleles deletion efficiency. Deletion band showed predicted 6 kb loss. In the presence of 8 mM L-glutamine all MAD7-edited clones showed doubling times comparable to WT cells (18–21 h). Under glutamine starvation Glul-deleted clones show no signs of proliferation contrary to WT cells. To mitigate the risk of unwanted pDNA genome insertion and add design flexibility we improve MAD7 gene editing system by optimizing crRNA delivery as a 2-O-Me synthetic oligo. Co-transfecting MAD7 mRNA with oligo-crRNA gives 20% less edit efficiency compared to crRNA-expressing pDNA in T7E assay due to rapid RNA degradation in cellulo before MAD7 protein is expressed. Delaying crRNA delivery 10h after MAD7 transfection resulted in 62% efficiency for both pDNA vector and synthetic oligo, while maintaining good cell viability and subsequent cloning efficiency. **Conclusions.** We developed full-RNA MAD7-based gene editing system and showed its ability to perform gene-size deletion of Glul in CHO-K1 cells, creating a host cell line suitable for GS-based metabolic stable selection.

Keywords: CRISPR, MAD7, crRNA, gene editing, glutamine synthetase, cell line engineering.

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