CRISPR/CAS9 AS A TOOL TO CORRECT POINT MUTATIONS IN A RECOMBINANT MONOCLONAL ANTIBODY GENE IN THE GENOME OF CHO CELLS.

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Obtaining high productivities of monoclonal antibodies requires the integration of multiple copies of the heavy and light chain genes into the genome of CHO cells. Here, a monoclonal antibody produced by a clonal cell bank of CHO cells was characterized by mass spectrometry. It was found that around 20% of the antibody had a sequence variant with an amino acid change in the heavy chain, making this high-producing cell line inadequate for commercial manufacturing. Characterization by next-generation sequencing (NGS) showed that a point mutation in one of the five integrated copies of the heavy chain gene was responsible for the amino acid change. We decided to repair the point mutation utilizing CRISPR/Cas9, which has been used along with single-stranded DNA oligonucleotides to increase the efficiency of point mutation repair in human-derived cell lines.[1] We designed a guide RNA that directs Cas9 specifically to the mutant base in the transgene. This guide RNA was cloned in the pspCas9 BB-2A-GFP vector under the U6 promoter, which also contains the Cas9 gene and EGFP as a reporter gene. The vector was transfected with an 80-base single-stranded oligonucleotide with the correct heavy chain sequence, as a template for the single-base repair. We isolated EGFP-positive single cells in 94 well plates and cultivated these clones. The genomic DNA from 16 clones was analyzed using a restriction enzyme that cuts only the corrected sequence of the transgene. A modified clone was selected, and the correct amino acid sequence of the therapeutic protein was confirmed by peptide mapping. With these results, we demonstrated that the CRISPR/Cas9 system is an alternative tool for the development of clonal cell lines to produce therapeutic proteins in CHO cells.

1. Bialk, P., Rivera-Torres, N., Strouse, B., & Kmiec, E. B. (2015). Regulation of Gene Editing Activity Directed by Single-Stranded Oligonucleotides and CRISPR/Cas9 Systems. *PLOS ONE, 10(6), e0129308.* doi:10.1371/journal.pone.0129308