Efficient Genome Editing of Mammalian Cells via Novel sgRNA Architectures and Cas9 Endonuclease

Alghamdi, Rania

10,000 of all known human diseases are monogenic, caused by a single mutation in the DNA. Scientists have tried developing the Zinc Finger Nuclease (ZFN) and Transcription Activator-like Effector Nucleases (TALEN) gene editing systems, but they required long term protein engineering and had high off-target activity. The CRISPR/Cas9 system has the ability to target the DNA at a specific site and generate a double stranded break, unlike ZFN and TALEN that target each strand of the DNA independently. Herein, a novel platform was developed based on modified short guide RNAs (sgRNAs) with different topologies to guide the Cas9 endonuclease for efficient editing in human cells was developed. In the experimentation, sgRNA strands, each complementary to the sense and antisense strand, respectively, were developed and then targeted on 3 different sites in the CCR5 and EMX1 locus. A repair template was inserted between left and right homology arms and then attached to the sgRNA. Subsequently, this was inserted into the DNA by taking advantage of the DNA's repair mechanism, homologous repair. The efficiency of the developed novel platform was tested on 40,000 HEK293FT cells by the insertion of a mutation in the 3 different target sites after successfully transfecting the cells with the plasmid encoding for the Cas9 gene and sgRNA. The platform was able to deliver the repair template to the target site by manipulating homologous repair. This will potentially allow for the future treatment of genetic diseases in humans, such as monogenic diseases, targeting the single mutation.

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