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Site-Directed RNA Editing by an Artificial Enzyme System

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RNA editing is a post-transcriptional process that produces various distinct functional proteins from a single gene. The molecular mechanism underlying C-to-U or A-to-I RNA editings are substitution of bases by the hydrolytic deamination of a cytosine or adenosine. Site-directed RNA editing is an important technique to correct genes and ultimately tuning protein function. We are trying to alter the genetic coded of transcripts(RNAs) by artificial RNA editing to treat genetic diseases. Adenosine deaminase acting on RNA (ADAR) family enzymes are usually programmed to target some specific adenosine governed by the upstream and downstream sequences. Here, we engineered the deaminase domain of ADAR1 enzyme and MS2 system to target specific adenosines to restore G-to-A mutations. For this purpose, upstream of the ADAR1 deaminase domain was fused with an RNA binding protein MS2, which binds to MS2 RNA. We designed a guide RNA complementary to target RNAs. Thus, the ADAR1 deaminase domain was carried to the desired editing site to convert adenosine to inosine. As a target, we mutated into amber (TAG) and ochre (TAA) stop codon at 58th amino acid Trp (TGG) of EGFP. In HEK293 cells, the above system could convert stop codons to read through and turn on fluorescence. We confirmed the specificity of editing by restriction fragment length polymorphism (RFLP) and sequencing; in protein level by western blot. The editing efficiency of this artificial enzyme system was up to approximately 5%. We think, this developed system could be used to treat diseases that result from G-to-A point mutation.

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References

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