Artificial RNA editing in mutated BFP (derivative of GFP) by using AID deaminase for restoration of genetic code

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Editing of mutated gene can be a possible means of treatment for genetic diseases caused by mutations in the normal codons. Here, I have tried to engineer deaminase domain of AID (Activated Induced Deaminase, a family member of APOBEC) and MS2 system to target specific Cytosine (C) to restore Thymine (T) that has been caused by the T to C mutations. For this catalytic domain of AID deaminase has been fused with RNA binding protein MS2, which binds to MS2-RNA. Guide RNA was designed complementary to target RNAs. Thus AID deaminase domain was carried out to desired editing site to convert C to U. As a target, Blue Fluorescence Protein (BFP) gene was prepared by mutating at 199 nucleotide of GFP. MS2 system has the ability to Convert Mutated Codon (CCA) to normal codon (CTA) in cellular system (e.g., HEK 293). The system converted CCA to CTA (conversion of cytidine to uridine transforms the BFP gene into the GFP gene) and turned on green fluorescence. cDNA was synthesized from positive cells followed by RNA extraction and PCR-RFLP was done by using BtgI restriction enzyme. The unedited BFP gave the fluorescence in the microscopic observation, remained uncut and edited were cut, also provide the green fluorescence expression by microscopic observation. Final confirmation was done by the Sanger's sequencing analysis where the restored one also gave the peak as the wild type "CTACGG" which was "CCACGG" in case of the mutated one (BFP).

Successful artificial editing of RNA in vivo by MS2 system can pioneer genetic code restoration therapy including stop-codon read through therapy for various genetic diseases.

Key words: RNA editing. AID, BFP, Guide RNA, Genetic code.

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