Abstract

Adenosine deaminase acting on RNA (ADAR) family enzymes consist of double-stranded RNA binding domains (dsRBDs) and a deaminase domain (DD) that convert adenosine (A) into inosine (I) acting as guanosine (G) during translation. Site-directed RNA editing is an important technique for correcting gene sequences and ultimately tuning protein function.

In this study, I engineered the DD of adenosine deaminase acting on RNA (ADAR1) and the MS2 system to target specific adenosines, with the goal of correcting G-to-A mutations at the RNA level. For this purpose, the ADAR1-DD was fused downstream of the RNA-binding protein MS2, which has an affinity for the MS2 RNA. I checked the binding affinity of artificial enzyme system by Biacore ™ X100. To direct editing to specific targets, I designed guide RNAs complementary to target RNAs. The guide RNAs directed the ADAR1 deaminase to the desired editing site, where it converted adenosine to inosine. To provide proof of principle, I used an allele of EGFP bearing a mutation at the 58th amino acid (TGG), encoding Trp, into an amber (TAG) or ochre (TAA) stop codon. In HEK-293 cells, this system could convert stop codons to read-through codons, thereby turning on fluorescence. I confirmed the specificity of editing at the DNA level by restriction fragment length polymorphism (RFLP) analysis and sequencing, and at the protein level by western blotting. The editing efficiency of this enzyme system was ~5%.

Further, I tried to compare the deaminase activity of ADAR1-DD and different isoforms of ADAR2-DD. The guide sequence was fused with MS2 stem-loop. As a target, mutated amber (TAG) stop codon at 58 amino acid (TGG) of EGFP was used. After transfection of the above three factors in HEK 293 cells, varying degree of the fluorescence signal was observed. Regarding ADAR2 isoforms, 120 bp consisting the Alu-cassette present in the middle of the DD. ADAR2-long without Alu-cassette showed much higher fluorescence signal than the ADAR2-long with Alu-cassette. According to the I-TASSER (Iterative Threading Assembly Refinement) data, inserted Alu-cassette result longer coil in the middle of the deaminase domain. Due to insertion of the Alu-cassette, the distance between residues after 203 is increased dramatically. The ligand binding site i.e. nucleic acid binding capacity also largely differ due to the insertion of the Alu-cassette. Another isoform ADAR2-short which is approximately 81 bp shorter at C-terminal, the fluorescence signal was undetectable. A single amino acid substitution of ADAR2-long (E488Q) renders the enzyme more active than the wild types. The fluorescence microscopic image and fluorescence spectra analysis are suggesting that ADAR1-DD is more active than ADAR2-long-DD. In the result of Western blot and sequencing, I found that ADAR1-DD is the most active deaminase than any other DDs. To my knowledge, this is a complete biological approach for the comparative study of ADARs-DD that gives important information on the rational use of DD in the future application for therapeutic purposes.

Regarding guide length, 21 bp guide found more functional and regarding the position, upstream guide is more efficient. The editing efficiency can be increased approximately 16%. Double mutated ochre (TAA) stop codon can be converted to (TGG) with 5' adenosine preference. Off-target editing increase with the increase of efficiency to the targeted adenosine. I believe that this system could be used to treat genetic diseases resulting from G-to-A point mutations.

Keywords- ADARs, MS2 RNA, RNA editing, stop codon, genetic diseases.