Abstract

The enzymes in the adenosine deaminase acting on RNA (ADAR) family have a deaminase domain (DD) that converts adenosine (A) into inosine (I), which functions as guanosine (G) during translation. In diseases caused by point mutations in genes, an important method for correcting RNA sequences and ultimately fine-tuning protein function is the artificial site-directed RNA editing.

In this study, I attempted to extend the MS stem-loop RNA to bind DD and guide RNA for the purpose of the editing efficiency of the MS2-ADAR1 RNA editing system. But the replacement of 6 X MS2 stem-loop RNA with 12 X MS2 stem-loop RNA was not valid due to the distance between the antisense part and the stem-loop part. My colleagues have developed a guide RNA that inserts stem-loops on both side of the complementary sequence of the target RNA, so I tried to improve the editing efficiency base on the 1-1 stem-loop guide RNA system. I increased the number of stem-loop on both sides and changed the paired base of the target nucleotide. The results showed that when the number of stem-loop on both sides was the same, the system showed high editing efficiencies in all conditions. In case of the paired base, when the paired base was U, the editing efficiency of this system was higher than other bases. These improvements might be very useful for treating genetic diseases that result from the G to A point mutation.

Our lab also developed the MS2-APOBEC1 system for restoring the T to C point mutation. It was used the deaminase domain of APOBEC1 (apolipoprotein B mRNA editing catalytic polypeptide 1) linked to the MS2 coat protein to perform the C to T deamination. I replaced the APOBEC1 catalytic domain with full-length human APOBEC3A and APOBEC3G. However, I can't detect any fluorescent signal from the cells transfected with the original guide RNA and APOBEC3A or APOBEC3G. I referred the natural substrate of APOBEC3A and APOBEC3G, so I designed the loop guide RNA for inducing loop structure on the target RNAs. I designed six types of guide RNAs to induce different lengths of loops for comparing the editing efficiencies. In all guide RNA conditions, I found that the 14nt loop guide RNA transfected with MS2-APOBEC3A or MS2-APOBEC3G could induce higher RNA editing efficiencies. I also used the D317W mutation of APOBEC3G transfected with the loop guide RNA. Even the D317W mutated APOBEC3G showed sequence preference to 5'-TC, the editing efficiencies were increased slightly. However, I couldn't detect any fluorescent signals from the cells that were transfected by the loop guide RNA and MS2-APOBEC1 system. For the application of guide RNA, it needs further optimization for improving the editing efficiency of the MS2-APOBEC system.

The proper application of the developed artificial deaminase system for the treatment of patients who have G-to-A or T-to-C mutations could open a new era in the field of treatment of genetic diseases.

Keywords: artificial RNA editing, base deamination, ADAR1, APOBEC, MS2 RNA