Abstract

Site-directed RNA editing is an importing technique for correcting gene sequence without requiring double-stranded DNA breaks. In previous research of our lab, artificial A-to-I RNA editing was done by using Adenosine Deaminase Acting on RNA (ADAR) and C-to-U editing enzyme of Apolipoprotein B mRNA Editing Catalytic Polypeptide-like (APOBEC) associated with guide RNAs complementary to the target sequence by MS2-tagged system, which have been successfully correcting G-to-A mutations and U-to-C mutation at the RNA level, respectively.

In my research, I am intended to investigate the potential of guanine deaminase (GDA) for restoring A-to-G mutations. Guanine deaminase (GDA), which is a ubiquitous enzyme that catalyses purine metabolism by hydrolytic deamination of guanine to xanthine (X) Xanthine is known to base pair with cytosine and thymine, in other words, it could be recognized as Guanine or Adenine. However, evidence for molecule or enzyme acting on G-to-X in RNA has not yet been found and whether guanine deaminase could complement to RNA catalysing G-to-X is also poor known. I apply GDA using MS2-tagged system to investigate the possibility of repair A-to-G mutation by targeting enhanced green fluorescent protein (EGFP). It is surprising that guanine deaminase, one of the most important subfamilies of deaminase comparing with ADAR, APOBEC, DYW, hardly anything is known about its analogous activity on introducing G-to-X on RNA or DNA. Therefore, I design dsRNA editing strategy, single strand RNA editing strategy and different editing sites to identify the GDA potential acting on G-to-X in RNA.

Additionally, I have introduced direct evolution for hADAR1 and hAPOBEC3A based on the protein-ligand docking results, to investigate whether specific mutants of ADAR and APOBEC3A have the potential of converting G-A. The constructed GDA plasmid for expressing MCP-GDA fusion protein to catalyse guanine to xanthine was confirmed, however, the transfection results of the MCP-GDA complex acting on EGFP mutant with A-to-G point mutation by multiple strategy of specific gRNA do not generalize any catalytic activity on RNA. Likewise, the transfection results of specific hADAR1 mutants and hAPOBEC3A mutants show no catalytic activity on RNA, suggesting that novel enzymes should be modified or discover towards G-to-A repair for gene therapeutic.

Key words: RNA editing, guanine deaminase, MS2-tagged system, G-to-X