ecRESCUE: a novel ecDHFR-regulated RESCUE system with reduced off-target activity

Yihan Wang

Guo Li

Xiangyang Li

Yuzhe Wang

Xingxu Huang

Xiaoxiang Hu

Jianen Gao

Video Byte

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Abstract

RNA base-editing tools enable scientists to study gene function and develop treatments for genetic diseases. The pioneering tool REPAIR changes A bases into I bases, while the newer RESCUE system changes A and C bases into I and U bases, respectively. However, both systems tend to edit unintended, or off-target, sites in addition to desired sites. To enable more precise RNA editing, researchers recently designed a new system, ecRESCUE, by fusing the destabilization domain (DD) of the *E. coli* protein ecDHFR to the RESCUE system. The DD degrades the two RESCUE proteins (dRanCas13b and ADAR2) unless the drug TMP is present, making the system drug-inducible. In vitro, ecRESCUE was just as effective as RESCUE in the presence of TMP, but ecRESCUE-mediated editing was greatly reduced in the absence of TMP. When 3 specific sites were targeted, TMP-induced ecRESCUE made many fewer off-target edits than RESCUE. Although ecRESCUE might need to be refined to further reduce or eliminate the remaining off-target effects, the results suggest that this new system is a promising improvement of the previous system and can achieve RNA base editing for gene function research with fewer unintended consequences.