INNATE PROGRAMMABLE DNABINDING BY Cas12m EFFECTORS ENABLES BASE EDITING

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The utilization of CRISPR technologies, namely Cas9 and Cas12a nucleases, has revolutionized genome editing. Yet, recognizing the drawbacks associated with current genome editing technologies, which rely on double-stranded breaks (DSBs), the attention is increasingly turning to alternative methodologies, such as base editing, to achieve precise and predictable modifications without DSB introduction. Adenine base editing, that involves the conversion of adenine (A) to guanine (G) within the DNA sequence, offers targeted and controlled single nucleotide modifications. Although adenine base editing activity has been demonstrated with the nuclease-impaired Cas9 and Cas12a effector proteins [1, 2], there is a demand for more compact protein variants that can retain the precision of base editing while being compatible with efficient packaging into adeno-associated virus (AAV) vectors, that are commonly used in clinical applications [3].

Cas12 effector proteins encoded in CRISPR-Cas type V systems are characterized by a wide range of sizes. They also contain relatively small Cas12m effector proteins (600 aa), the size of which would not limit the use of AAV vectors to deliver protein-encoding genes. Previously, we demonstrated that Cas12m proteins provide protection against bacteriophages and plasmids through targeted DNA binding rather than DNA cleavage. Therefore, this innate programmable DNA binding could offer an alternative for Cas9 and Cas12 protein-based base editing.

This work aimed to test Cas12m innate DNA binding ability to be adopted for programmable base editing. We engineered the potential base editors by fusing selected Cas12m proteins with adenine deaminase TadA-8e. Their activity was evaluated by testing the targeted A-to-G editing in human cells. Overall, this work contributes towards the attempts to develop a new generation of base editing tools compatible with AAV vectors.

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