Investigating the use of Xeno Nucleic Acids (XNAs) in guide-strand design for RNA-editing by ADAR

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***Abstract*:**

Adenosine deaminases acting on RNA (ADARs) are a family of enzymes that catalyze the deamination reaction of Adenine (A) to Inosine (I) in dsRNA. ADAR1 and ADAR2 perform the catalytic conversion of Adenine (A) to Inosine (I) in humans. The X-ray crystal structure of the human ADAR2 deaminase domain bound to double stranded RNA reveals the flipping of the target Adenosine (A) into the enzyme active site, which facilitates the ADAR editing. The structure also reveals how ADARs bind to the A-form double helical structure found in dsRNA. Chemical modification of the guide RNA improves the RNA editing rates and lowers the off-target RNA editing. In this regard we have designed a chemically modified guide RNA composed of XNA and RNA which exhibits ADAR1 specific A to I RNA editing in-vitro. Unlike regular unmodified guide the newly designed guide RNA shows minimal off target editing while maintaining high RNA editing efficiency for the target Adenosine (A). The guides designed were tested for different target sequences and exhibit similar patterns of editing efficiency while maintaining minimal off target editing. Kinetic assays performed show almost similar rates as the control guide RNA. Preliminary in-vivo RNA editing assays performed in HEK293T cells shows promising editing in HEK293T cells.

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