Tunable Control of Base Editors

Adeeb Alomar, BS; Andreas Chi, BS; Ning Liu, PhD; Rhonda Bassel-Duby PhD; Eric Olson PhD.

UT Southwestern Medical Center

UT Southwestern Medical Center

Background

CRISPR-Cas genome editing tools continue to lead new advances in possible therapeutic treatments for genetic diseases. One class in particular, the base editor, is a fusion protein of a Cas nickase and a deaminase, which are directed by a sgRNA to allow targeted base pair conversion. The viral delivery of the construct leads to episomal structures that lead to life-long expression of the cargo transgene, which may allow long-term promiscuous activity of the base editor[1]. Destabilizing Domains (DD) are short protein sequences that are inherently unstable under physiological conditions. When DDs are fused to a protein of interest, the entire fusion protein is rapidly degraded by the proteasome. However, addition of a small molecule ligand can rapidly stabilize the fusion protein and prevent degradation.

Aim

Design a DD-Base Editor fusion protein that allows tunable and controllable expression in vitro using a small molecule ligand.

Methods

Modified E. coli dihydrofolate reductase was selected as a DD that can be stabilized with trimethoprim (TMP). Various configurations of DD-linked base editors were constructed, packaged into lentivirus, and infected into HEK293T cells. Tunable protein expression and DNA editing efficiency were explored by Western blot and DNA sequencing, respectively.

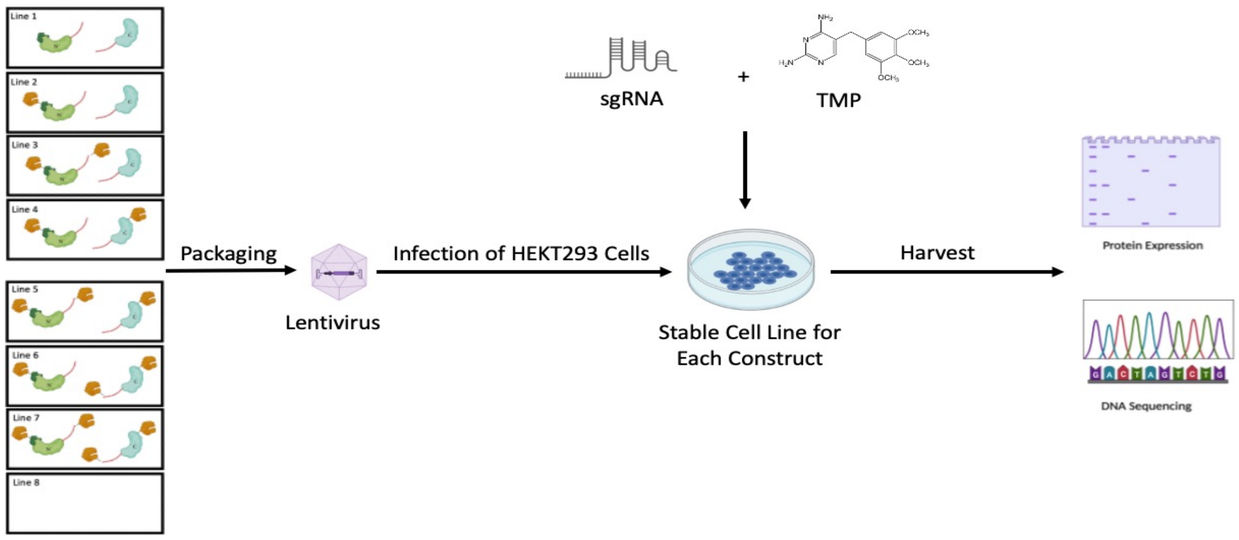

Figure 1. Experimental Design.

Results

Constructs [DD-N + C] and [DD-N + C-DD] showed modulated protein expression in response to the presence of TMP. DNA editing efficiency in the absence of TMP for these constructs were comparable to background, whereas levels in the presence of TMP were similar to our positive control with no destabilizing domains.

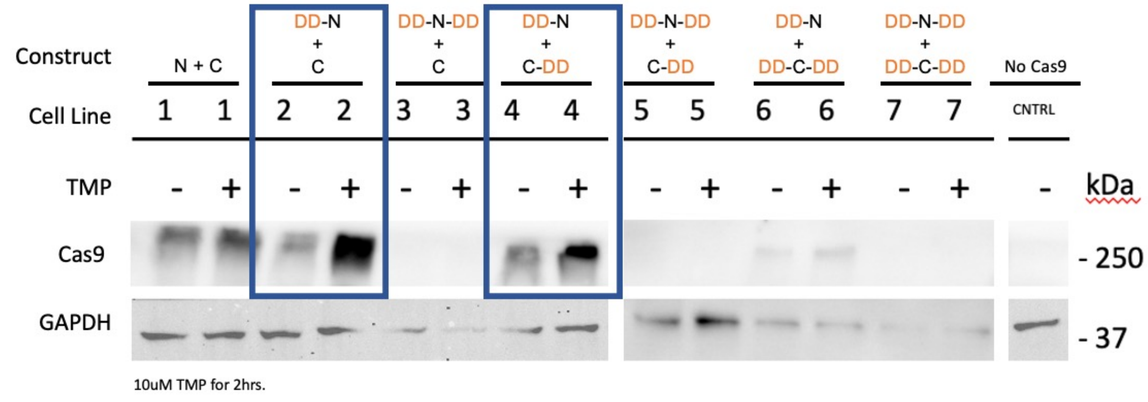

Figure 2. Cas9 protein expression after 2hrs exposure to 10uM TMP.

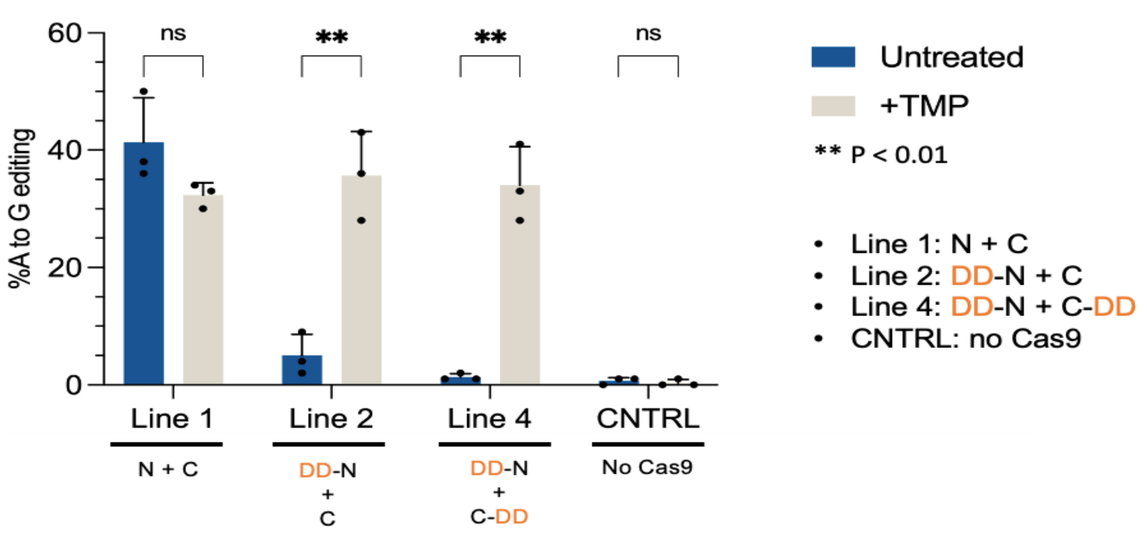

Figure 3. Cas9 protein function after 2hrs exposure to 10uM TMP.

Conclusions

DD-Base Editor constructs are a promising tool that allows tunable control of base editor protein expression. The cloning configuration of the DD is important in predicting the efficiency and functionality of the final base editor protein. Further directions include studying in vivo mice models and long-term efficiency of the constructs.

References

Penaud-Budloo M, Le Guiner C, Nowrouzi A, Toromanoff A, Chérel Y, Chenuaud P, Schmidt M, von Kalle C, Rolling F, Moullier P, Snyder RO. Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle. J Virol. 2008 Aug;82(16):7875-85. doi: 10.1128/JVI.00649-08. Epub 2008 Jun 4. PMID: 18524821; PMCID: PMC2519600.