Targeting MSH3 for Triplet Repeat Diseases Treatment using CasPlus

Ursula Andreo*, Menggui Huang*, Lozen Robinson, Ryan Gaudlip, Ryshone Duncan, Sapanna Chantarawong, Rebecca Haley and Ravi Iyer.

ABSTRACT :

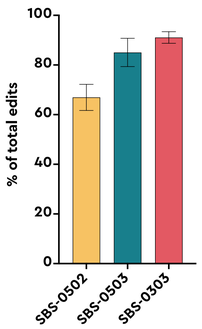
Huntington's disease (HD) and Myotonic dystrophy type 1 (DM1) are progressive disorders caused by CAG and CTG repeat expansions in the *HTT* and *DMPK* genes, respectively. MSH3, a DNA mismatch repair gene, has been identified as a genetic modifier of disease onset/severity of both HD and DM1, and drives repeat expansion in animal models of disease. Therefore, MSH3 has emerged as an attractive therapeutic target for slowing down HD and DM1 progression by attenuating repeat expansion. Although no MSH3-targeted therapies are in the clinic, ongoing approaches have focused on chronic reduction of MSH3 function either by RNAi-mediated gene silencing or small molecule inhibition. We are developing a novel "one-and-done" CRISPR-based gene knockout strategy for targeting MSH3 in the brain. Our approach utilizes two proprietary technologies: (i) the CasPlus gene editing system that can generate +1 frameshift knockout mutations with higher efficiency and safety than conventional CRISPR approaches, and (ii) a novel lipid nanoparticle (LNP)-based delivery ATHENA™ (**A**dvanced **T**echnology for **H**ighly **E**fficient **N**eural **A**dministration) platform that can deliver RNA therapeutics to the brain, with minimal off-target accumulation in the liver, spleen or peripheral organs.

Herein we demonstrate that CasPlus-optimized single guide RNAs (sgRNAs) targeting hMSH3 result in 70-90% knockdown efficiency in Hek293 cells as judged by DNA sequencing, immunoblot and immunofluorescence. Furthermore, we have developed and optimized a highly sensitive clinic-ready assay to detect hMSH3 protein levels using the Meso Scale Discovery Platform (MSD). Packaging these CasPlus and sgRNA components with CNS-specific ATHENA™ LNPs results in >70% editing, paving the way for validating in HD and DM1 pre-clinical mouse models. Script ATHENA™ LNPs are also efficient to package hMSH3 targeting siRNA which could facilitate their delivery to the brain.

To test our therapeutic *in vivo* in mice, we have also designed CasPlus-optimized single guide RNAs (sgRNAs) targeting mMSH3 that generate >70% of +1 frameshift mutations at the target locus in NIH3T3 cells. Script's innovative Cas-Plus platform and CNS-specific LNP technology offer a promising strategy to address unmet medical needs in triplet repeat diseases like HD and DM1, which could complement therapies currently being developed by other biopharmaceutical companies.

Editing profile in human Hek293 cells

A.



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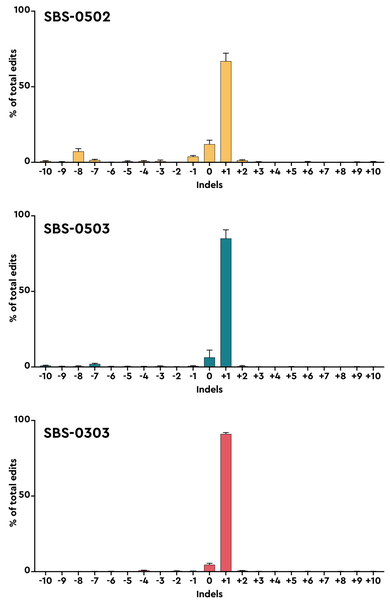
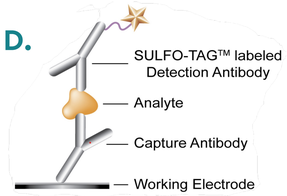
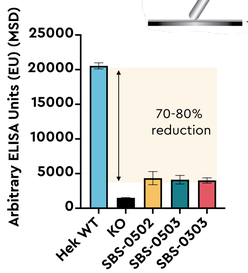


MSH3 Protein Levels Meso Scale ELISA

C.

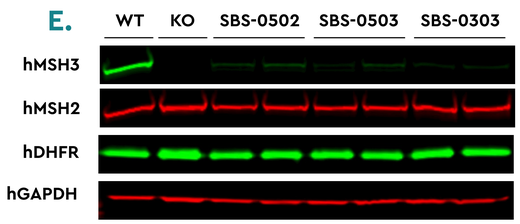
D.





hMSH3 expression in Hek293 cells

E.



F. Immunofluorescence showing hMSH3 expression in Hek293 cells

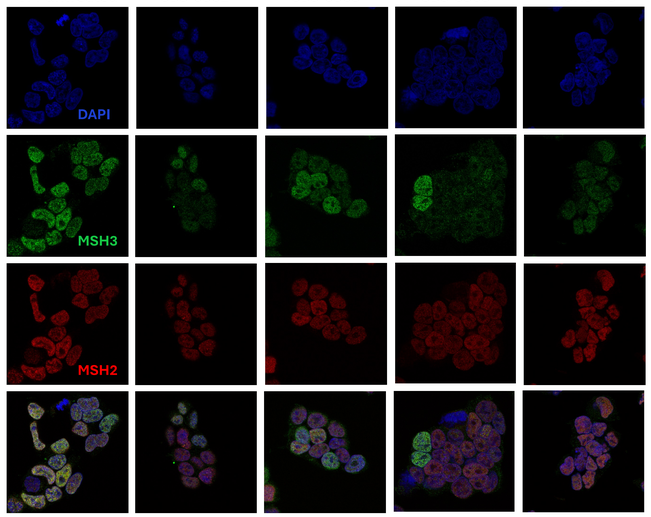
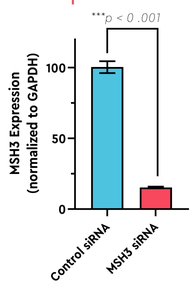


Figure 1 : Cas-Plus editing of hMSH3 in Hek293 cells
Hek293 cells were electroporated with CasPlus and 3 hMSH3 specific guides (SBS-0501, SBS-0503 or SBS-0303). After 72h, genomic DNA was extracted, amplified by PCR and the sequence was analyzed by Sanger and TIDE. The editing profile showed a majority of +1 frameshift **(A)**. A representative result of editing -10 to +10 nucleotide around the editing site for each single guide is shown **(B)**. Editing resulted in the reduction of at least 70% of hMSH3 protein expression using the Meso Scale ELISA technology illustrated **(C&D)**. Western blot showed very efficient knock-down (70 to 80%) of hMSH3 while neither hMSH2 nor DHFR expression were affected **(E)**. Electroporated Hek293 cells were stained for MSH3, MSH2, DHFR and DAPI after 72h and representative immunofluorescent images are shown **(F)**.

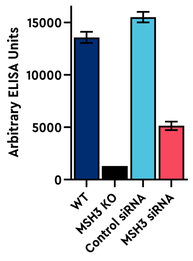
MSH3 mRNA levels qPCR

A.



MSH3 Protein Levels Meso Scale ELISA

B.



MSH3 Protein Levels Immunoblot

C.

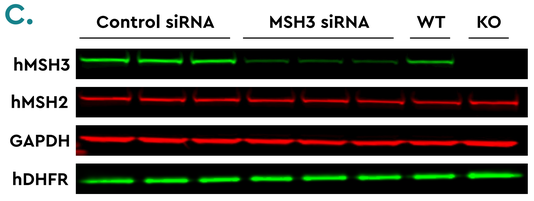
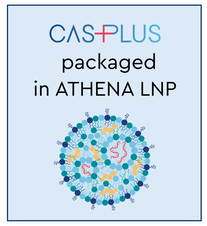
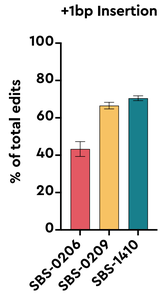


Figure 2 : ATHENA™ mediated delivery of hMSH3 targeting siRNA in Hek293 cells
Hek293 cells were treated with 200ng siRNA per 50,000 cells for 72h. Cell RNA and proteins were extracted and hMSH3 mRNA was quantified using quantitative PCR normalized by GAPDH mRNA indicating 90% of MSH3 mRNA expression after siRNA treatment **(A)**. MesoScale ELISA showed reduction by 75% of hMSH3 protein expression **(B)** and is consistent with the Immunoblot results **(C)**.

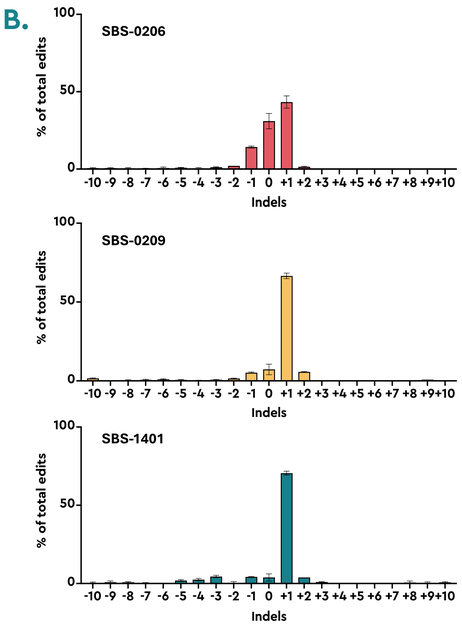
Editing profile in mouse cell lines C2C12 and NIH-3T3



A.



B.



MSH3 Protein Levels Immunoblot

C.

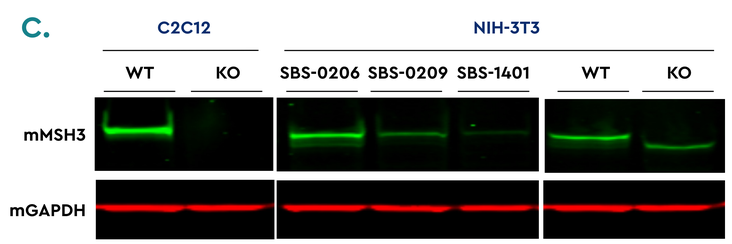


Figure 3: Cas-Plus editing of mMSH3 in NIH-3T3 cells
NIH-3T3 cells were treated with CasPlus and 3 different guides SBS-0206, SBS-0209, and SBS-1410. DNA was extracted, amplified by PCR and the sequence was analyzed by Sanger and TIDE. The editing profile showed a majority of +1 frameshift **(A)**. A representative result of editing -10 to +10 nucleotides around the editing site for each single guide is shown **(B)**. Western blot showed very efficient knock-down over 70% of mMSH3 especially for SG10 **(C)**.

CONCLUSION/PERSPECTIVES :

We have identified human and mouse sgRNAs targeting MSH3 which exhibit high editing efficiency *in vitro*. Editing is specific and generates a majority of a +1 frameshift leading to an efficient knockdown of MSH3 without affecting the expression of the overlapping gene DHFR.

The CasPlus system can be efficiently packaged in Script Biosciences LNP ATHENA™ and can efficiently reduce MSH3 expression making it an attractive therapeutic strategy to target triplet repeat diseases like Huntington and DM1 especially in the brain.

Cas-Plus reference :
T4 DNA polymerase prevents deleterious on-target DNA damage and enhances precise CRISPR editing. Yang Q., Abebe JS, Mai M, Rudy G, Kim SY, Devinsky O, Long C. EMBO J. 2024 Sep;43(17):3733-3751. PMID: 39039289

See also: Script Biosciences Oral presentation AMA 1824
by Menggui Huang
Oral Abstract Session Lipid Nanoparticles II
Location: Room 393-396 Friday
16th 1:30 PM - 3:15 PM

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