Sending out an SSO

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Introduction

FRDA Repeat Expansion

- Neurodegenerative disease with autosomal recessive inheritance
- Caused by repeat expansion in the first intron of the FXN gene
- Disease severity is directly linked to repeat tract length
- Repeats lead to deficiencies in mitochondrial protein frataxin
- Frataxin is responsible for energy production
- Characterized by loss of voluntary muscle movement, loss of positional and vibration sense, slurred speech, and loss of reflexes

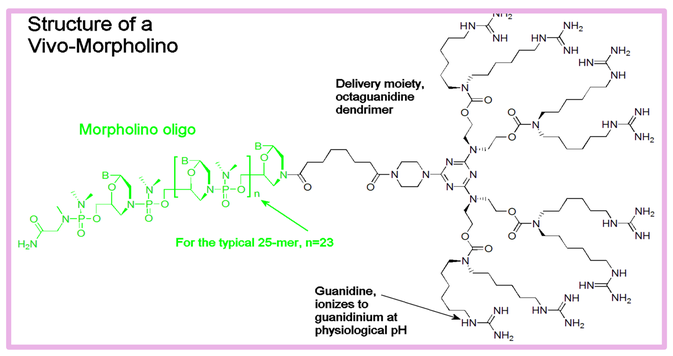
Cardiac Effect

- Repeats expand to greatest lengths in the heart
- Cardiomyocytes require substantial amounts of energy to function
- Frataxin deficiencies cause energy deficiencies
- Most FRDA patients develop cardiomyopathy which results in heart failure and death

Objective

- In this study, we attempt to establish a model for peptide-mediated oligonucleotide (SSO) delivery into cardiac cells
- We aim to determine the most effective orientation for cell penetration through analysis of the N terminal (N-Tat) and C terminal (C-Tat) of the peptide

Methods



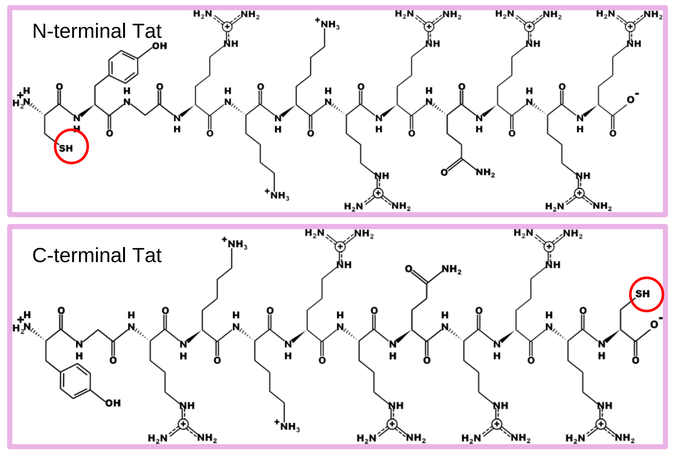


Figure 1: Vivo Morpholino, HIV-1 N-terminal Tat, and HIV-1 C-terminal Tat structures ordered top to bottom. HIV-1 N and C terminal Tat differ by SH group in structure that binds to oligonucleotide.

Binding SSO to induce MLH3 splice redirection to isoform 2

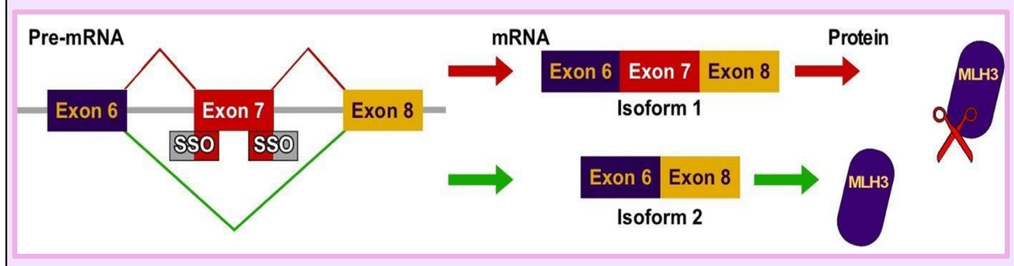


Figure 2: SSO bound to RNA exon induces MLH3 splice redirection from splice variant 1 to splice variant 2. SSO directs pre-mRNA splicing by binding to the sequence surrounding exon 7 and inducing a splice redirection. MLH3 isoform 1 cutting mechanism facilitates further DNA repeat expansion. MLH3 isoform 2 does not have cutting mechanism, thus, inhibiting repeat expansion.

Results

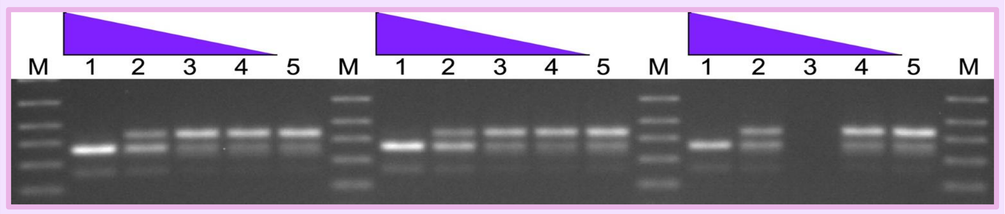


Figure 3: MLH3 Vivo-Morpholino positive control MLH3 splice direction. HEK 293 cells dosed at 3.16µm, 1.0µm, 0.316µm, 0.1µm, and 0µm of positive control vivo morpholino. RNA analysis performed 24 hours following treatment. Triangle represents dose from high to low (3.16µm, 1.0µm, 0.316µm, 0.1µm, and 0µm). MLH3 splice variant 1 shown at 534 bp and splice variant 2 at 462 bp. (72 bp difference). M is a 1kb plus DNA ladder showing 650 bp, 500 bp, 400 bp, 300 bp, and 200 bp bands.

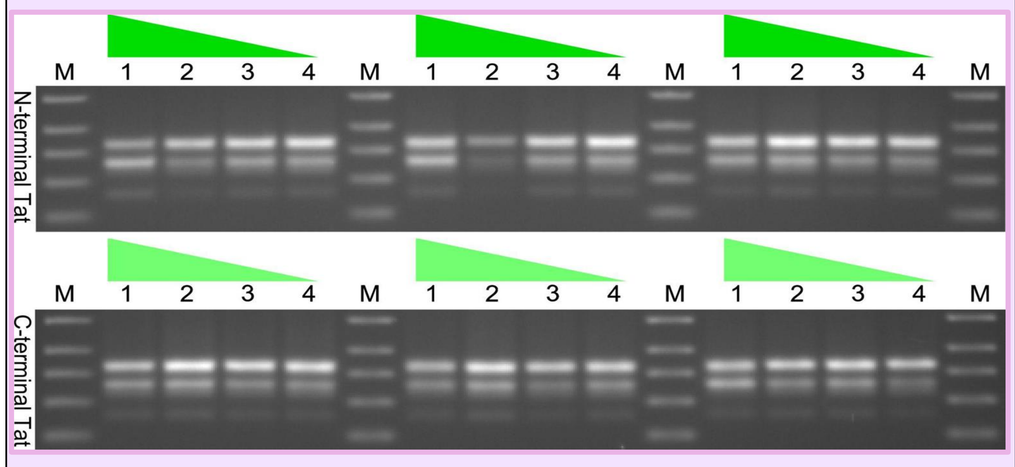


Figure 4: MLH3 HIV-1 N-Tat-SSO and C-Tat-SSO splice direction. HEK 293 cells dosed at 3.16µm, 1.0µm, 0.316µm, and 0.1µm of HIV-1 N-Tat (top gel) and C-Tat (bottom gel). RNA analysis performed 24 hours following treatment. Triangle represents dose from high to low (3.16µm, 1.0µm, 0.316µm, and 0.1µm). MLH3 Splice variant shown at MLH3 splice variant 1 shown at 534 bp and splice variant 2 at 462 bp. (72 bp difference). M is a 1kb plus DNA ladder showing 650 bp, 500 bp, 400 bp, 300 bp, and 200 bp bands.

Conclusions

HEK cells treated with Vivo Morpholino

- Vivo Morpholino shows successful cell penetration as expected
- Gel electrophoresis analysis shows a dose-dependent increase in the amount of MLH3 splice redirection to isoform 2

HEK cells treated with N-tat-SSO and C-tat-SSO

- HIV-1 N-Tat and C-Tat show successful penetration
- Unexpected difference in the amount of MLH3 splice redirection between N-Tat and C-Tat
- N-Tat linkage appears more effective than C-Tat

Future Directions

- The long-term goal is to slow or even stop somatic repeat expansion in the heart, preventing the progression of cardiomyopathy and death in FRDA patients
- Many studies operating with HIV-1 Tat peptide conjugate drug through the C terminal, however, our findings show more efficacy through the N terminal
- The next steps are to test and compare the efficacy of the SSO bound to the N terminal and C terminal of a cardiac targeting peptide (CTP)

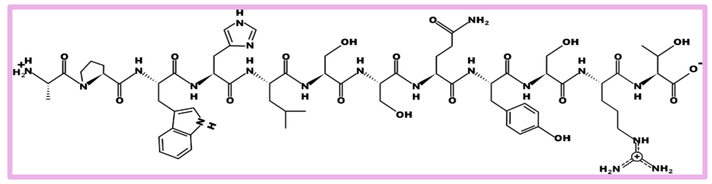


Figure 5: Cardiac Targeting Peptide (CTP) Structure. CTP is designed to target cardiomyocytes with minimal uptake in other tissues such as the kidney and brain. the peptide has been shown to function in vitro and in vivo with mice models.

- Preliminary steps will be taken in vitro, future directions involve a mouse model for SSO bound to CTP testing

References

1. Images (figure 1 and 5) generated by Pepdraw, Wimley Lab, Tulane University School of Medicine, Department of Biochemistry. https://pepdraw.com/
2. Roy, J. C.L., Vitalo, A., Andrew, M. A., et al. (2021, March 22). Somatic CAG expansion in Huntington's disease is dependent on the MLH3 endonuclease domain, which can be excluded via splice redirection. Nucleic Acids Research, 49(7), 3907- 3918. doi:10.1093/nar/gkab152
3. Zahid, M., Phillips, B. E., Albers, S. M., Giannoukakis, N., Watkins, S. C., & Robins, P. D. (2010, August 17). *Identification of a cardiac specific protein transduction domain by in vivo biopanning using a M13 phage peptide display library in mice*. PloS one. https://pubmed.ncbi.nlm.nih.gov/20808875/