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"Sending out an SSO"

Abstract

Friedreich Ataxia (FRDA) is a progressive neurodegenerative disease caused by repeat expansion in the first intron of the FXN gene. FRDA is lethal, with the leading cause of death being heart failure due to repeat expansion. The number of repeats is directly proportional to disease severity. The repeats expand to the greatest length in the heart. If we can stop repeats from expanding in the heart, we could slow or even stop the progression of cardiomyopathy. Expansion leads to reduced expression of the FXN gene, which encodes the mitochondrial protein frataxin, responsible for iron-sulfur cluster formation and is essential to energy production. Cardiac cells require substantial amounts of energy to keep the heart pumping. Consequently, deficiencies in frataxin lead to cardiovascular problems in addition to neurological problems, as neurons and cardiomyocytes require the most energy.

Our long-term goal is to slow or even stop somatic repeat expansion in the heart. My project takes preliminary steps to establish a model for peptide mediated delivery. The DNA mismatch repair complex MutLγ (MLH1-MLH3) is responsible for DNA repeat expansion. MutLγ containing isoform 1 of MLH3 produces expansion and MutLγ containing isoform 2 of MLH3 does not. To slow repeat expansion, we aim to splice-switch MLH3 isoform 1 to MLH3 isoform 2. Oligonucleotides can be designed to induce splice redirection. Successful oligonucleotides are called Splice Switching Oligonucleotides (SSO). Our lab has developed an SSO that can successfully induce splice redirection. In this study, as a prelude to testing a cardiac targeting peptide, we linked the SSO to a well-known cell penetrating peptide (CPP), HIV-1 tat, to penetrate our model cells, HEK 293. As a positive control we used the vivo morpholino variant of the same sequence. We compared the Vivo, N-tat and C-tat linked versions. We isolated RNA 24 hours after dosing and determined MLH3 splice-switching by PCR product analyzed via gel electrophoresis. HEK 293 cells treated with a Vivo-SSO showed successful penetration and a dose-dependent increase in the amount of MLH3 splice-switched to isoform 2 as expected. HEK 293 cells treated with N-tat-SSO and C-tat-SSO show splice-switch to isoform 2 in high doses, providing encouraging information for future research.