Name(s)

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Project Number

S0505

Project Title

RT-qPCR Development of a New Therapeutic Avenue for Curing Amyotrophic Lateral Sclerosis (ALS)

Abstract

Objectives

The suppression of Ataxin-2 leads to decreased TDP-43 protein aggregates in mice brains that showed Amyotrophic Lateral Sclerosis symptoms. The purpose of this study is to develop a reliable high-throughput qPCR method to measure quantitative decreases of Ataxin-2 RNA levels in human U2OS cells, ultimately to screen for a small molecule drug screen to reduce TDP-43 aggregates in ALS patients.

Methods

U2OS human cells were seeded in a 384-well format and transfected with either non-targeting siRNA or Ataxin-2-targeting siRNA. Cells were washed and lysed. RNA was reverse transcribed to cDNA in a 96-well plate following a SYBR Green Kit protocol. qPCR was run with the cDNA and unique primer pairs for Ataxin-2 and GusB. The qPCR Ct values were analyzed using double delta ct method and graphed; statistical significance was calculated by t-test. Surfaces and equipment were cleaned with 70% ethanol and Thermo Fisher RNaseZap.

Results

After testing different siRNA conditions and primer pairs in a standard 12-well qPCR setup, there was a ~50% decrease between Ataxin-2 siRNA treated cells and non-targeting siRNA treated cells with 0.0028 p-value. Testing a high-throughput qPCR setup in a 384-well format using the Life Technologies Cells to Ct kit showed no significant decrease and high variability in the levels of Ataxin-2 RNA, with 0.55 p-value. Testing different variables within the kit s protocol such as the reverse transcription cell lysate input also showed an insignificant p-value and decrease. Changing to the BioRad Singleshot SYBR Green kit, there was a ~50% decrease between the non-targeting and Ataxin-2 siRNA, with a 0.0015 p-value.

Conclusions

After testing multiple conditions and seeing no decrease in Ataxin-2 siRNA transfected samples, the Life Technologies kit is not viable for high-throughput screening, and will not be used for any future experiments. After observing a significant p-value and decrease in Ataxin-2 RNA with the Bio-Rad kit, future experiments will be pursued using this kit and the primer pairs tested in the low-throughput setting. The results from this project will be applied to screening FDA approved drugs to identify compounds that decrease Ataxin-2 RNA levels. These drugs will later be tested in in vitro cell models of ALS and an ALS mouse model to further investigate its effect on TDP-43 aggregation in ALS patients.

Summary Statement

This project optimizes a RT-qPCR method of testing Ataxin-2 RNA levels in U2OS cells to ultimately apply towards drug testing in ALS patients.

Help Received

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