Identifying Compounds for the Treatment of Spinocerebellar Ataxia Type 2 (SCA2)

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Introduction

SCA2 is an autosomal dominant cerebellar ataxia characterized by progressive degeneration of the cerebellum and brain stem. SCA2 is caused by polyglutamine expansion in the SCA2 protein ataxin-2, and is characterized by gain of normal or toxic function. Based on partial reversibility of phenotypes after transgene silencing in polyQ models and lack of major deleterious effects upon *ATXN2* knockout in the mouse, we sought to identify compounds that reduce *ATXN2* expression.

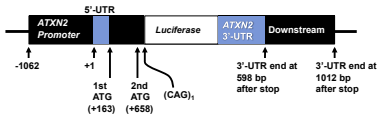
Objectives

To identify a small molecule-based treatment for SCA2 that reduces total ataxin-2.

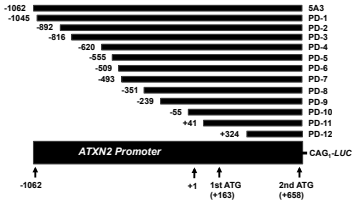
Methods

- We established a cellular model for high throughput compound screening (HTS) consisting of HEK293 cells constitutively expressing *ATXN2* exon1 up to the 1st CAG, fused in-frame to luciferase under control of the *ATXN2* promoter, and including the *ATXN2* 3'-UTR and polyA signal.
- We conducted a robotically controlled HTS of 65,000 compounds, including 1120 FDA approved drugs.
- Screening was accomplished by plating 6000 cells/well in 384 well plates to wells preplated with 10 μM compounds. Cells were incubated 24 hrs then assayed for luciferase.
- Orthogonal, secondary and viability screening was accomplished in 384 well plates with two compound doses of 1 and 10 μM, then repeated in 96 well plates for selected compounds using 12 comopund doses from 0 – 10 μM.

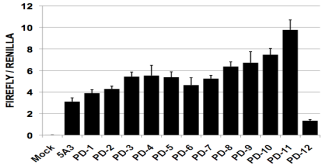
***ATXN2* reporter construct: plasmid pGL2-5A3**



***ATXN2* promoter analysis: promoter deletions made in pGL2-5A3**

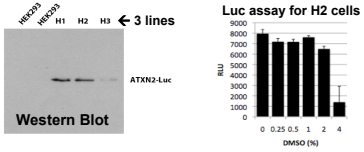


Increasing luciferase expression from pGL2-5A3 with increasing promoter deletion: evidence for control elements for expression specificity.

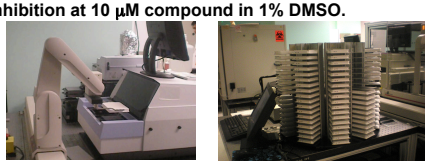


***ATXN2-Luc* expression *in vivo* was verified in 5A3 transgenic mice**

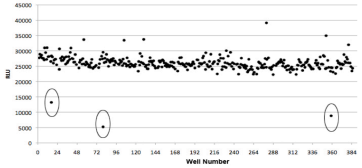
***ATXN2-Luc* is expressed in stably transfected HEK293 cells and uneffected by 1% DMSO**



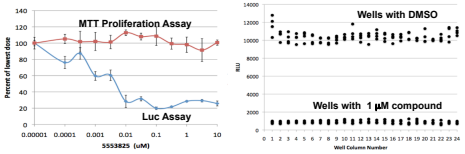
65,000 compounds were screened robotically for *ATXN2-Luc* inhibition at 10 μM compound in 1% DMSO.



Example of one 384 well plate assay showing three positive hit compounds.



***Z'*-Factor calculation was conducted using the strong *ATXN2-Luc* inhibitor ChemBridge 5553825 at 1 μM. The calculated *Z'*-factor is 0.804.**
(*Z'*-factor = $1-[(3(\sigma_{exp}+\sigma_{cont}))/|\mu_{exp}-\mu_{cont}|]$).



Orthogonal Screening

Compounds were tested for *ATXN2-Renilla* Luc inhibition using compound doses 1 and 10 μM.

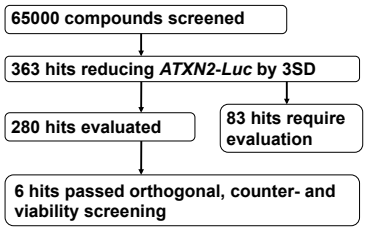
Counterscreening

Compounds were tested for *CMV-Luciferase* inhibition using compound doses 1 and 10 μM.

Viability Screening

Compounds inhibiting proliferation in MTT assays were eliminated from further consideration.

Results



Discussion

Previous studies demonstrated that reductions of mutant proteins for HD, SCA1 and SCA3 in inducible mice were associated with reversals of disease phenotypes (Yamamoto *et al.* 2000; Zu *et al.* 2004; Boy *et al.* 2009). Additionally, non-allele specific silencing of the SCA3 gene (*MJD1*) reduced neuropathology in a rat model (Alves *et al.*, 2010). Thus, targeting total ataxin-2 may be an effective strategy for the treatment of SCA2.

Conclusions

- We determined that the *ATXN2* promoter specifically drives *ATXN2*-luciferase expression in mouse cerebellum.
- We identified six compounds that may prove to be effective therapeutics for SCA2 by down-regulating *ATXN2* expression, and we are in the process of evaluating other positive hits.
- We will continue by determining *ATXN2-Luc* inhibition in transgenic mice and ability for compounds to ameliorate SCA2 phenotype in mouse models of the disease.

Acknowledgements

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Disclosures: none.