An AI-predicted chemical compound suppresses ATXN1 protein aggregation and rescues iNSC-derived SCA1 neurons from cell death

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Background

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by CAG repeat expansions in the ATXN1 gene. The mutant ataxin-1 (ATXN1) protein forms toxic oligomers which slowly aggregate into larger insoluble inclusions within the nucleus. The AXH domain of ATXN1 is suggested to play a critical role in the aggregation of its mutant isoform. Currently, there is no treatment for SCA1.

Aims:

- Identification of chemical compound which would bind to the AXH domain and might suppress its dimerization and polyQ-expanded ATXN1 aggregation
- Generation and characterization of iNSC-derived neurons from SCA1 patients
- Validation of the AI-predicted novel compound in aggregation and cell death on iNSC-derived SCA1 neurons

Identification of novel compound suppressing ATXN1 dimerization

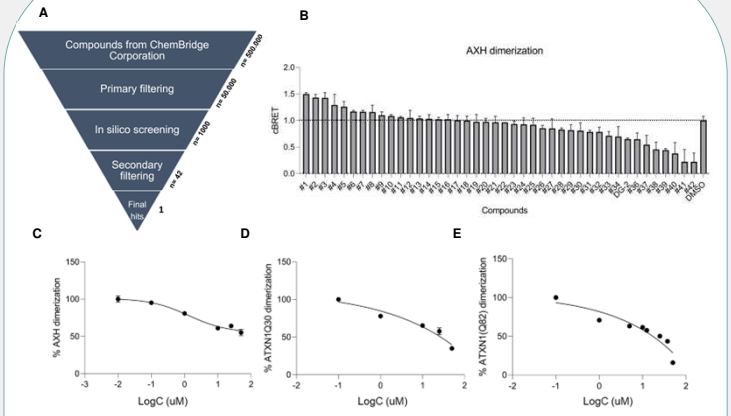

Figure 1. (A) **In silico screening workflow** for the identification of compounds binding to the AXH domain. **(B)** LuTHy assay for the AXH dimerization in the presence of predicted compounds. At least **17 compounds** suppress AXH dimerization. **(C,D,E)** Dose-dependent effect of compound **DG-2**. This compound suppresses AXH and ATXN1 (wild-type and mutant) dimerization in a dose-dependent manner.

In vitro effects of DG-2 compound in a cellular model of mutant ATXN1 aggregation

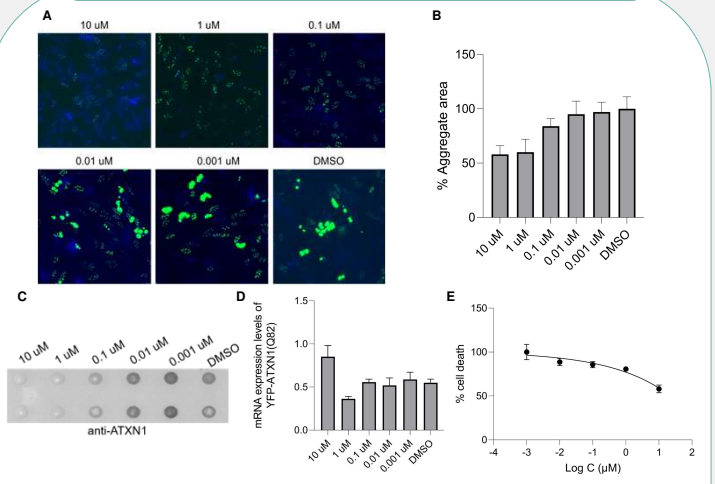

Figure 2. (A,B) DG-2 compound **suppresses** the formation of **insoluble ATXN1(Q82) protein inclusions** in a dose-dependent manner. **(C)** No insoluble material was observed in cells treated with high concentrations of DG-2 as shown by filter retardation assay. **(D)** DG-2 does not affect the expression of YFP-ATXN1(Q82) transgene. **(E)** The **reduction** in insoluble poly-Q expanded ataxin-1 aggregates directly correlates with the suppression of **caspase 3/7 activity.**

Generation of iNSC-derived mature neurons

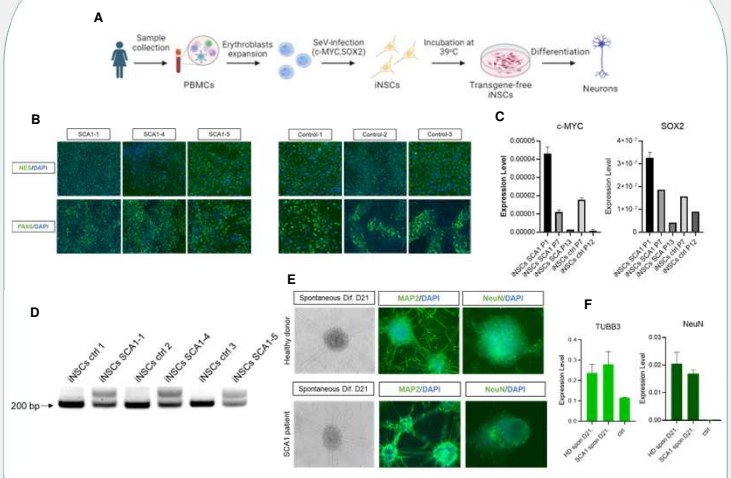

Figure 3. **(A)** Trans-differentiation of PBMCs from three SCA1 patients and three age/sex matched healthy donors into **induced neural stem cells (iNSCs)**. **(B)** iNSCs exhibit neural lineage markers (NES and PAX6), **(C)** are virus-free **(D)** and maintain the expected patient genotype. **(E,F)** After 6 weeks of spontaneous differentiation, iNSCs give rise to **mature neurons**, marked by positive staining for MAP2 and NeuN. (HD: healthy donor). Error bars denote ± SD.

Neuroprotective effects of DG-2 in SCA1 neurons

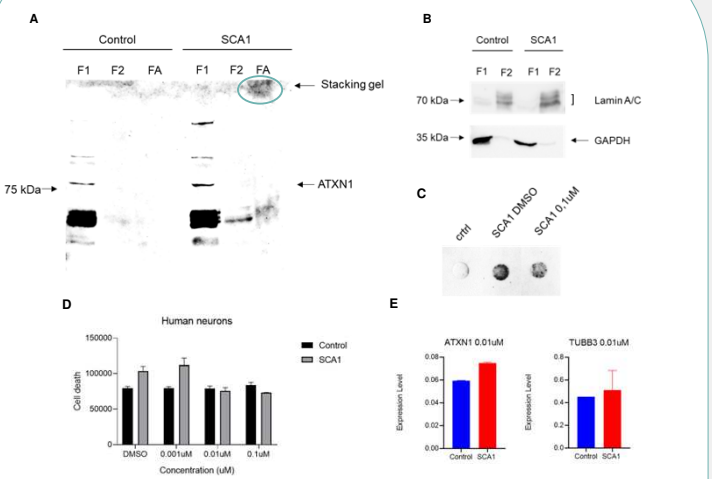

Figure 4. **(A,B)** Accumulation of **ATXN1-positive insoluble inclusions** in iNSC-derived SCA1 neurons. **(C)** Filter retardation assay further confirmed the presence of insoluble inclusions of polyQ-ATXN1 in extracts from SCA1 neurons. DG-2 reduces the accumulation of these insoluble aggregates in SCA1 neurons. **(D)** Treatment with DG-2 shows **neuroprotective effects** by reducing programmed cell death in SCA1 neurons. **(E)** Expression levels of ATXN1 and TUBB3 in both SCA1 and control neurons remained similar after treatment with DG-2. Error bars denote ± SD.

Distribution of DG-2 in blood and cerebellum of CD-1 mice

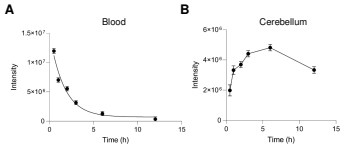

Figure 5. LC-MS/MS analysis shows DG-2 transfer from blood to cerebellum, with **(A)** blood levels decreasing as **(B)** cerebellum levels peak around 6 hours, indicating **blood-brain barrier crossing**.

Conclusions

DG-2 compound:

- binds to the AXH domain of ATXN1 and inhibits its dimerization
- reduces the aggregation of ATXN1 inclusions in Tet-On YFP-ATXN1(Q82) SH-SY5Y cells & iNSC-derived SCA1 neurons.
- rescues iNSC-derived SCA1 neurons from cell death

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