Comparative Analysis of *In Vitro* Systems for TDP-43 Pathology

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Background

Recent regulatory clearances utilizing induced pluripotent stem cells (iPSCs) underscore the need for novel translational models to facilitate drug testing in neurodegenerative disorders. Among these, the transactive response (TAR) DNA-binding protein 43 (TDP-43) stands out, given its role in gene regulation and its implication in conditions such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), and Parkinson's disease (PD). Evidence suggests that TDP-43 dysfunction, characterized by mislocalization, hyperphosphorylation, and aggregation is associated with cytoplasmic stress granule (SG) formation, which in turn affects RNA translation during cellular stress. Cryptic splicing and polyadenylation events in genes like Stathmin-2 (STMN-2) and UNC13A have been observed in tissues from patients with ALS, FTD, and AD. Therefore, we have developed multiple in vitro models mimicking TDP43 pathology in human cell systems.

Methods

Human TDP-43 overexpressing neuroblastoma cells and iPSC-derived glutamatergic as well as motor neurons carrying the M337V mutation in the TARDP gene were used. These models were exposed to the stress granule inducers sodium arsenite (SA) or MG-132 and treated with potential therapeutic agents such as edaravone and riluzole. TDP-43 translocation and stress granule formation were analyzed using immunocytochemistry. Cryptic splicing of STMN-2 and UNC13A was assessed using polymer chain reaction (PCR).

Results

STMN-2 Functional mRNA Expression in Different Cell Types

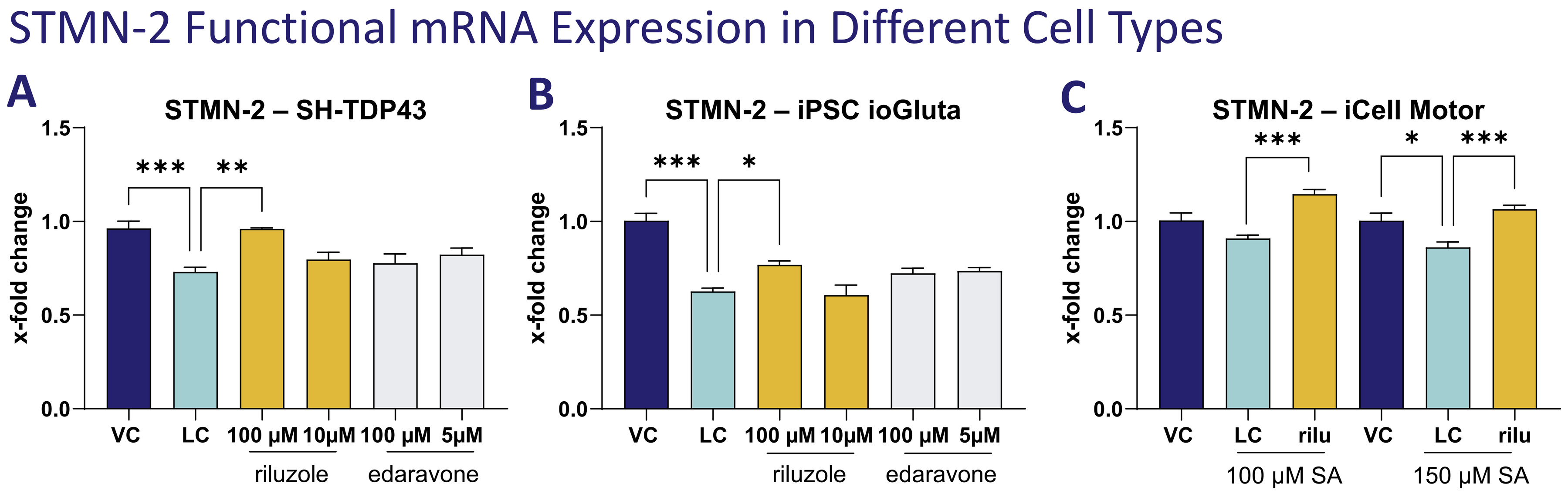

Figure 1: Effect of riluzole and edaravone treatment on STMN-2 functional mRNA expression in different SA-lesioned cell types. SH-TDP-43 cells (**A**) or iPSC-derived ioGluta neurons (**B**) were pretreated with riluzole or edaravone for 24h and then lesioned with 100 µM SA. iPSC-derived iCell motor neurons (**C**) did not respond as strong to SA as the other cell types and were therefore treated with 100 as well as 150 µM SA in presence or absence of riluzole. Thereafter, mRNA expression of STMN-2 was analyzed by qPCR. Data are given as x-fold change of RNA expression in relation to vehicle controls (VC). Mean + SEM; n = 6-8 per group; One-way ANOVA followed by the Dunnett`s *post hoc* test versus lesion control (LC); *p <0.05, **p <0.01, ***p <0.001.

Alternative mRNA Splicing in SH-TDP-43 and iPSC-Derived ioMotor Neurons Cells

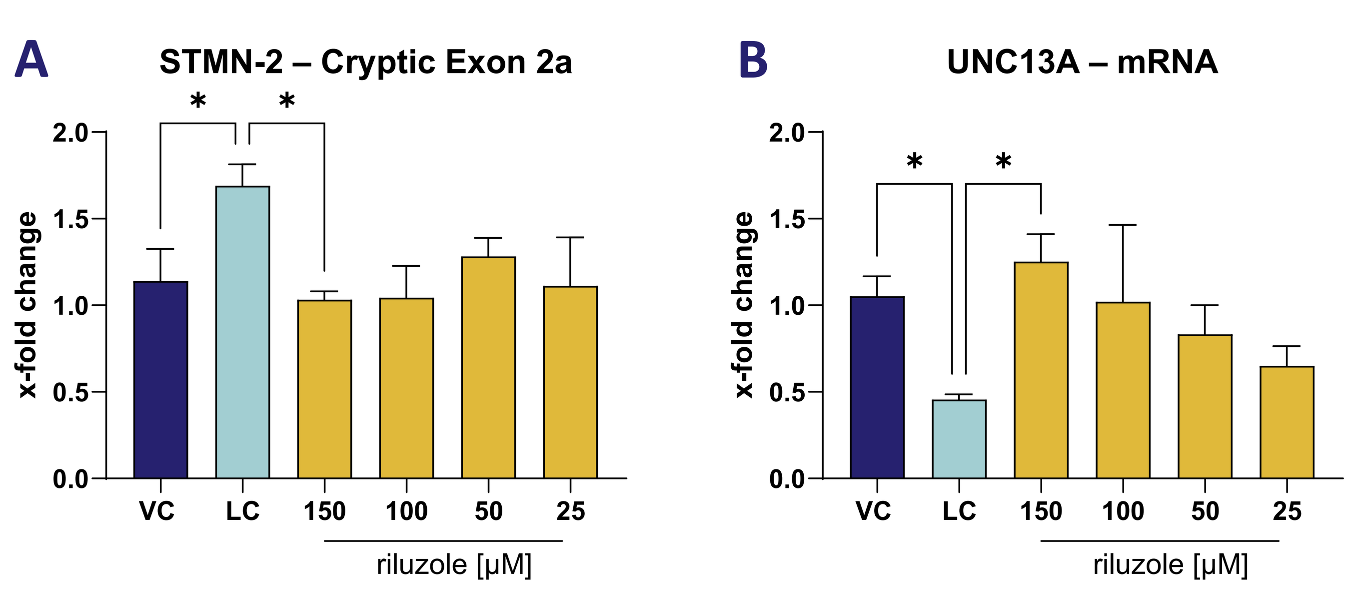

Figure 2: Effect of riluzole treatment on STMN-2 cryptic exon 2a and UNC13A functional mRNA expression in SA-lesioned SH-TDP-43 cells. Cells were pretreated with riluzole at multiple concentrations for 24h and then lesioned with 100 µM SA. Thereafter, mRNA expression of STMN-2 CE 2a (**A**) as well as functional UNCA13A (**B**) was analyzed by qPCR. Data are given as x-fold change of RNA expression in relation to vehicle controls (VC). Mean + SEM; n = 6 per group; One-way ANOVA followed by Dunnett`s *post hoc* test versus lesion control (LC); *p <0.05.

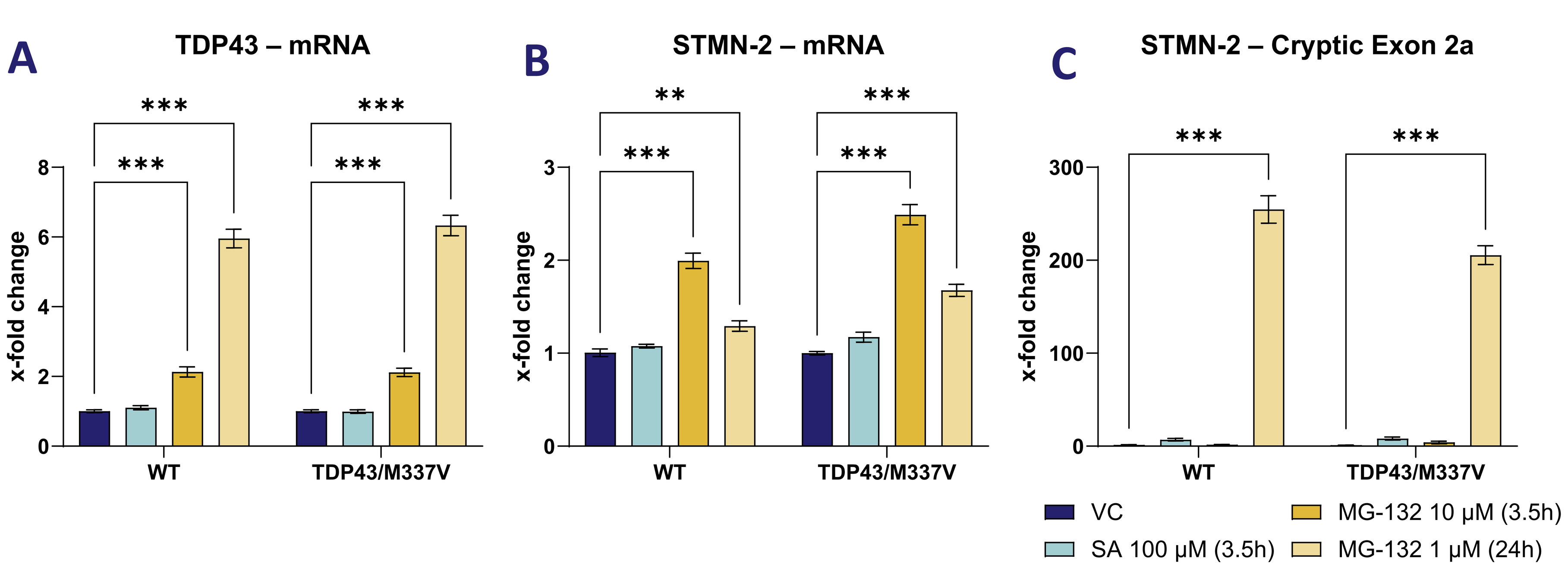

Figure 3: Expression levels of TDP43 and STMN-2 in wild type (WT) control and TDP-43 M337V/WT ioMotor neurons. Cells were lesioned with 100 µM SA and 1 µm MG-132 for 3.5h and 10 µM MG-132 for 24 hours. Thereafter, mRNA expression of TDP43 (**A**), functional STMN-2 (**B**) and STMN-2 CE 2a (**C**) was analyzed by qPCR. Data are given as x-fold change of RNA expression in relation to vehicle controls (VC). Mean ± SEM; n = 6 per group; One-way ANOVA followed by Dunnett`s *post hoc* test versus lesion control (LC); **p <0.01; ***p <0.001.

Results

TDP-43 Translocation and Stress Granule Formation in SH-TDP-43

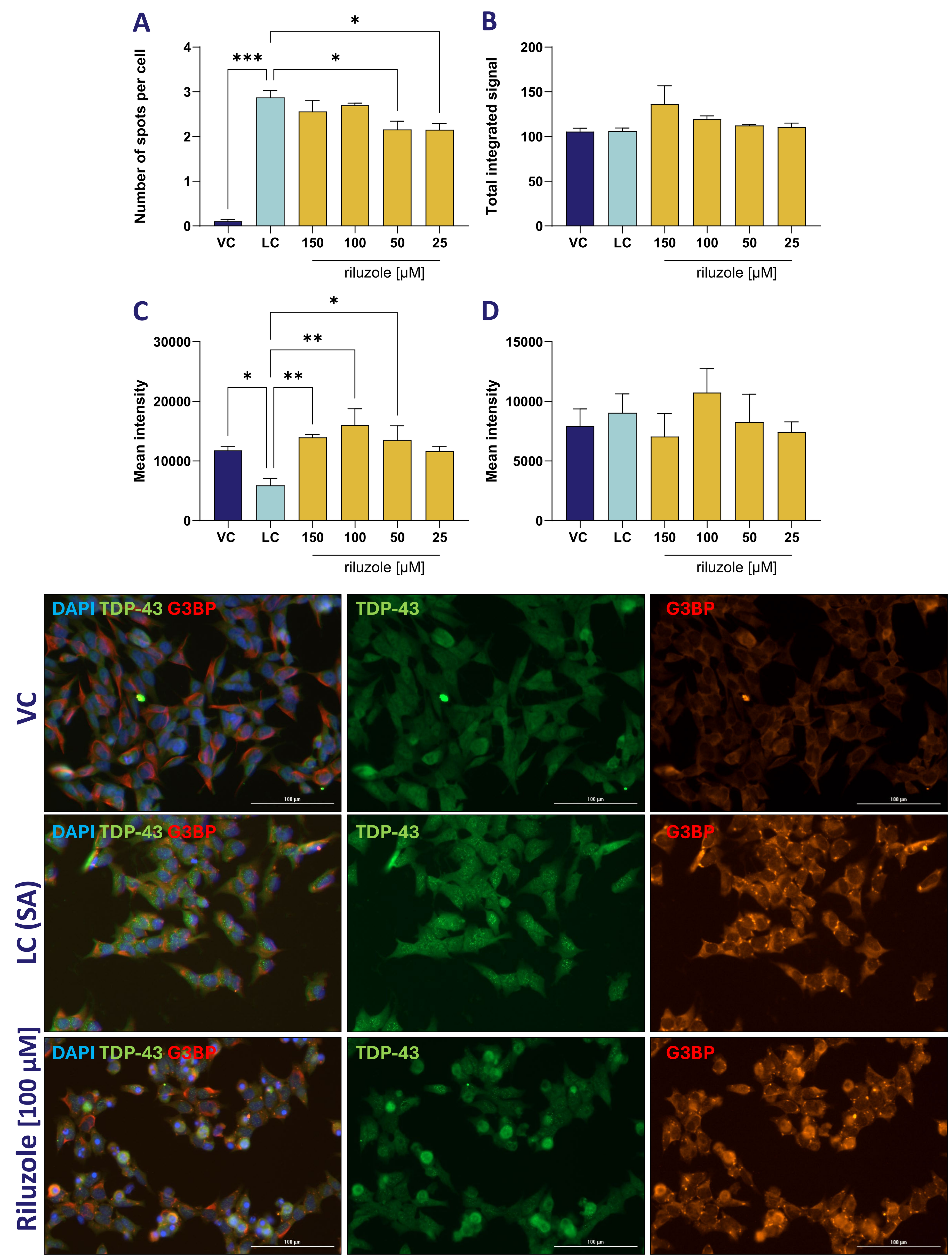

Figure 4: Stress granule formation as well as TDP-43 levels and localization in SA-lesioned SH-TDP-43 cells after riluzole treatment. Cells were pretreated with riluzole for 24h and then lesioned with 100 µM SA and assessed for stress granules as G3BP-positive spots per cell via ICC (**A**) as well as, total TDP-43 signal (**B**) and nuclear (**C**) versus cytosolic (**D**) TDP-43 intensity. Additionally, representative images of unlesioned vehicle control (VC), SA-lesioned control (LC) and SA-lesioned and riluzole-treated cells are shown. Data are given as percent of unstimulated VC (**A**) and signal intensity (**C, D**) or total signal (**B**). Mean + SEM; n = 4-6 per group; One-way ANOVA followed by the Dunnett`s *post hoc* test versus LC; *p <0.05, **p <0.01 and ***p <0.001.

Summary

In conclusion, our *in vitro* models utilizing SA-induced SGs provide valuable platforms for screening the efficacy of investigational compounds targeting TDP-43-related pathologies and downstream effects. These models offer insights into the molecular mechanisms underlying neurodegenerative diseases and pave the way for the development of novel therapeutic interventions.

For more information about the models please visit: www.scantox.com
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