Stress-induced nucleocytoplasmic shuttling of TDP-43 is controlled by eIF-5A hypusination

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Introduction

Aggregation and phosphorylation of TAR DNA-binding protein-43, TDP-43, has been found to be associated with the neuropathology of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTLD). It has been proposed that TDP-43 accumulation in stress granules (SG) may contribute to the aggregation of TDP43.

Eukaryotic translational initiation factor 5A (eIF5A) is hypusinated by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). It is involved at the level of mRNA turnover, cell proliferation, and protein translational elongation. In this experiment we sought to determine the function of hypusinated eIF5a in relation to TDP-43 pathology in nuclear and cytoplasmic compartments under cellular stress.

Hypothesis

When hypusination of eIF-5A is inhibited there is decreased levels of TDP-43 aggregation in the cytoplasm.

Methods

Tet was characterized by plating HeLa cells on a 96 well plate and reading GFP fluorescence on a Cytation 3 Cell Imager (Excitation max 395 nm, Emission max 509 nm) every 15 minutes for 24 hours after addition of varying tetracycline concentration (1, 3, 10, 30 µg/mL).
GC7 dose response

For the effects of eIF5A hypusination inhibition, HeLa Q331K cells were grown in petri dishes for. After 24-hour incubation, tetracycline was added to the petri dishes (except for control). The next day, cells were treated with 0 or 50 µM of GC7 and incubated for 72 hours. Cells were then treated with 0 or 10 µM of sodium arsenite for one hour. Thereafter, cells were lysed and a subcellular fractionation was performed to isolate cytoplasmic and nuclear fractions. Western blot technique was then used to measure levels of TDP-43 and pTDP-43. Stress granule levels in the cytoplasm were measured with TIA-1, PABP, and G3BP. eIF5A and eIF-5A^{HYP} levels were also measured.

Experimental Design

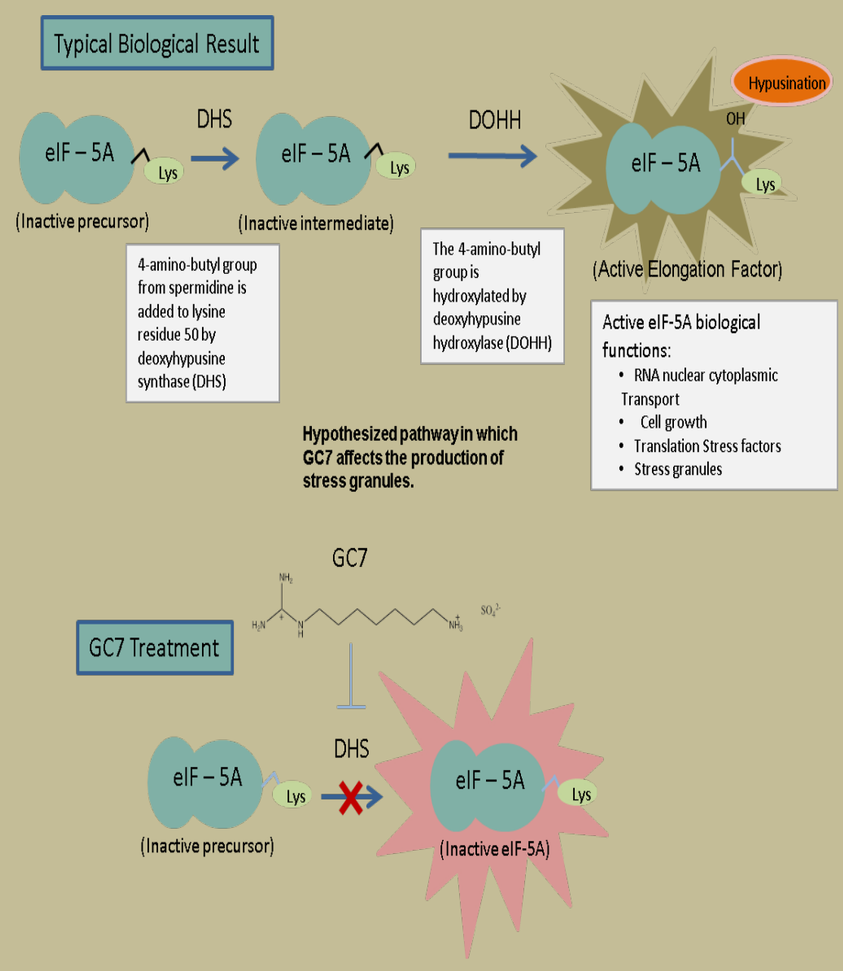


Figure 1.

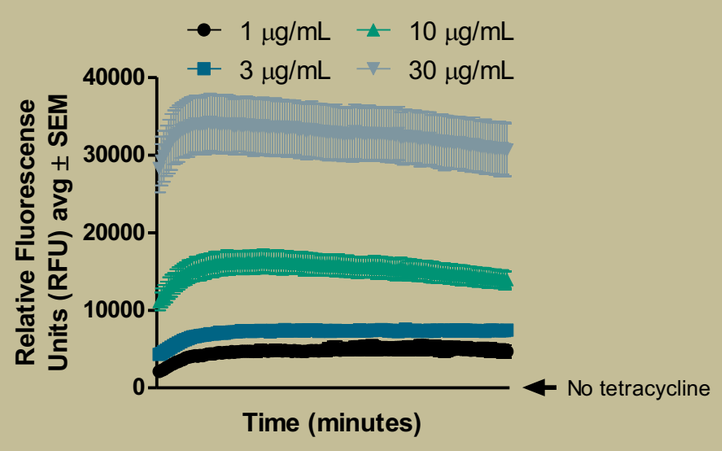


Figure 2.

Figure 3.

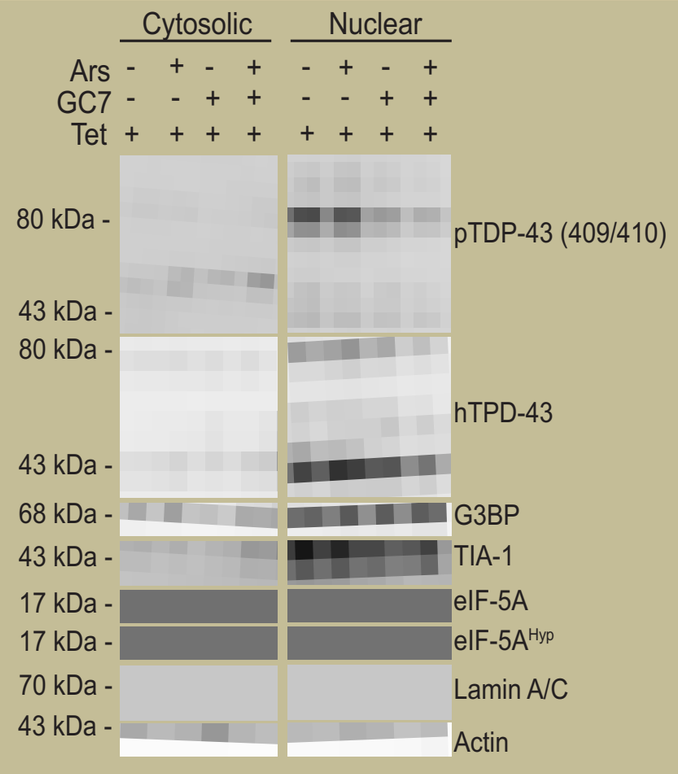
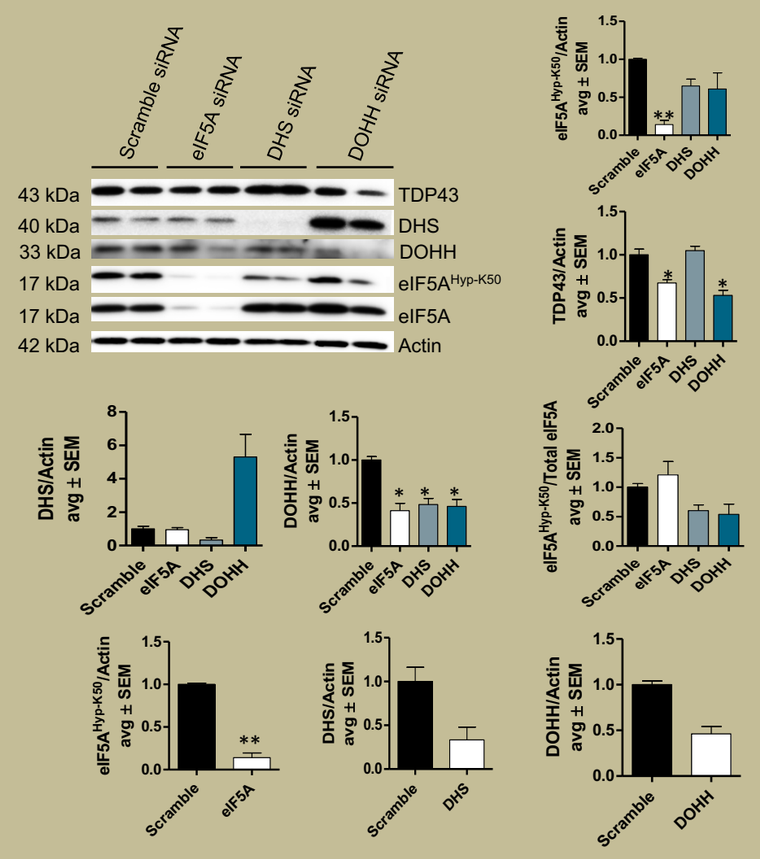


Figure 4.



Conclusion

An optimal concentration of GC7 and tetracycline was established. Additionally, by inhibiting hypusination of eIF-5A, TDP-43 nucleocytoplasmic shuttling and stress granule formation was reduced.

Future Directions

- Future experiments can be done to look at the effects of a DOHH inhibitor and compare to it to GC7 inhibition.
- The same experiment will be performed on wildtype HeLa cells