Effects of ALS-associated mutations on the in vivo aggregation and toxicity of human FUS/TLS protein

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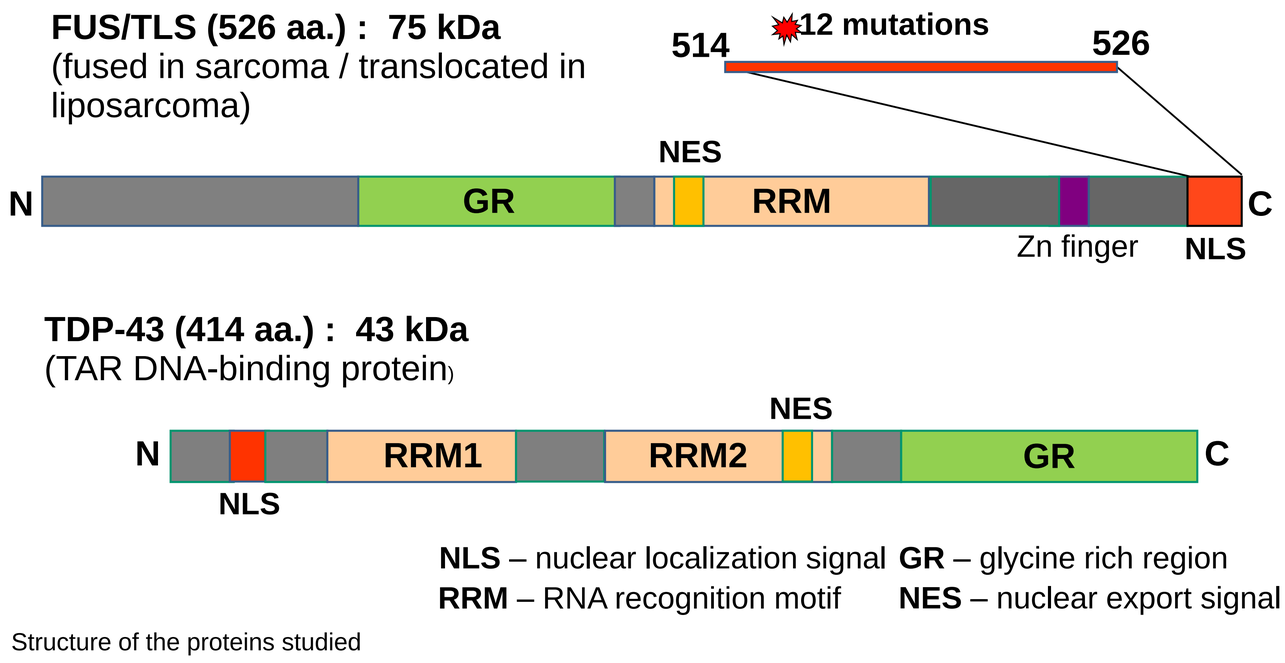
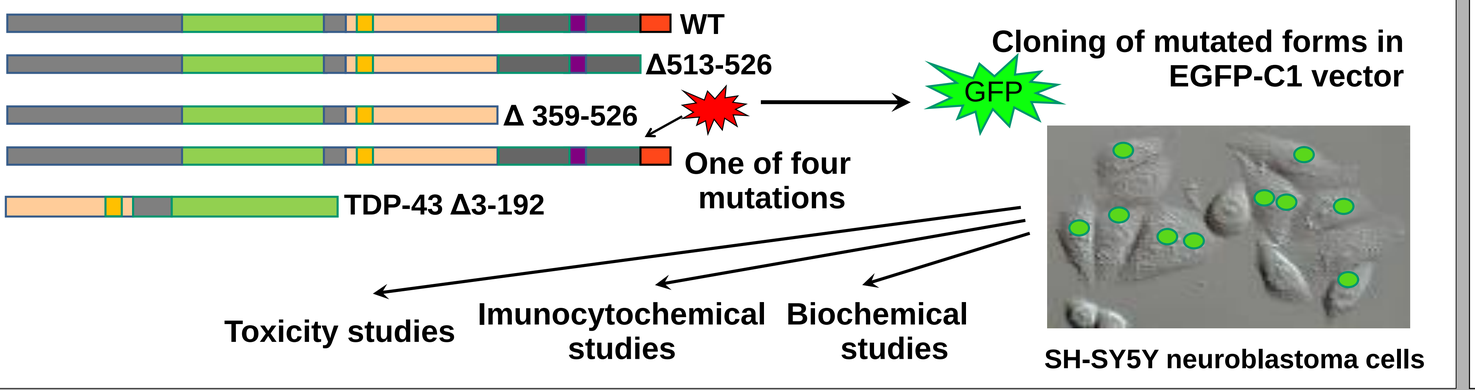
Introduction

Multiple mutations in two genes encoding proteins involved in the intracellular RNA metabolism, maturation and transport, transactive response (TAR)-DNA-binding protein 43 (TDP-43) and fused in sarcoma/translated in liposarcoma (FUS/TLS), have been associated with the development of familiar and sporadic forms ALS and FTLD-U. Moreover, both TDP-43 and FUS/TLS or their truncated forms were identified as major constituents of characteristic intracellular inclusions in the neuronal and glial cells of patients with familiar and sporadic forms of ALS and FTLD-U. However, neither the mechanism of these proteins aggregation nor the consequences of inclusion formation to brain cell physiology is known. To study the effect of FUS/TLS mutations associated with human neurodegenerative disorders on aggregation and toxicity of this protein in vivo we employed a cellular model system, SH-SY5Y human neuroblastoma cell lines expressing wild type or various mutated and truncated forms of FUS/TLS as eGFP fusion proteins.



Structure of the proteins studied



Mutation	Protein distribution: nuclear diffuse	nuclear aggregates	cytoplasmic diffuse	cytoplasmic aggregates
WT (full-length)	+	+	-	-
R518L	+	+	-	-
R524T	+	+	-	-
R525L	-	+	-	+
R522G	-	-	+	+
Δ513-526	-	-	-	+
Δ359-526	-	-	-	+
TDP-43/Δ3-192	+	+	+	+

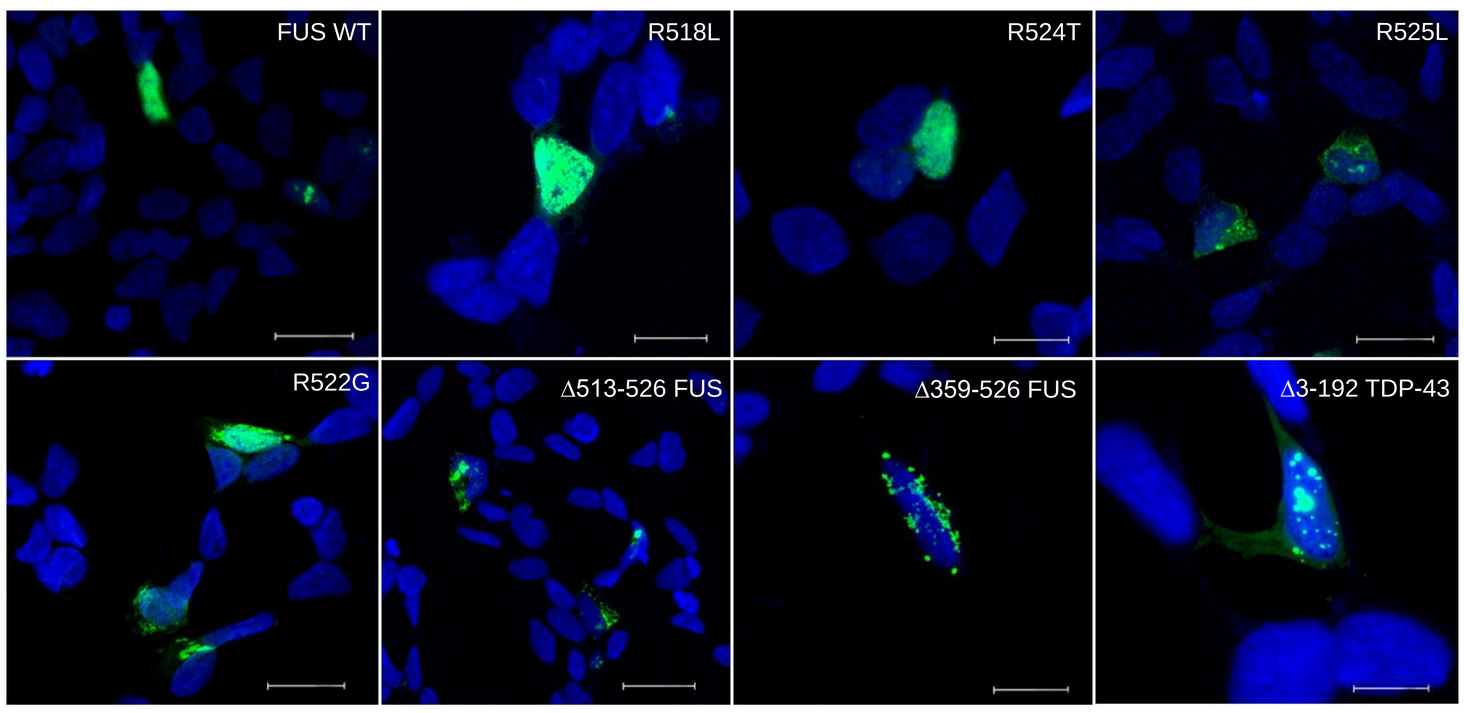


Figure 1 Distribution of TDP-43 and FUS/TLS and morphological types of aggregates formed by these two proteins in SH-SY5Y neuroblastoma cells upon transfection with corresponding construct. Green – protein+GFP; blue – DAPI.

Time point	Number of cells with lost adhesion ability: Empty vector, cells per 0.1 μL±SEM	FUS/TLS Δ359-526, cells per 0.1 μL±SEM
24 h	7.08 ± 0.78	8.50 ± 0.92
48 h	6.92 ± 0.71	14.33 ± 1.63

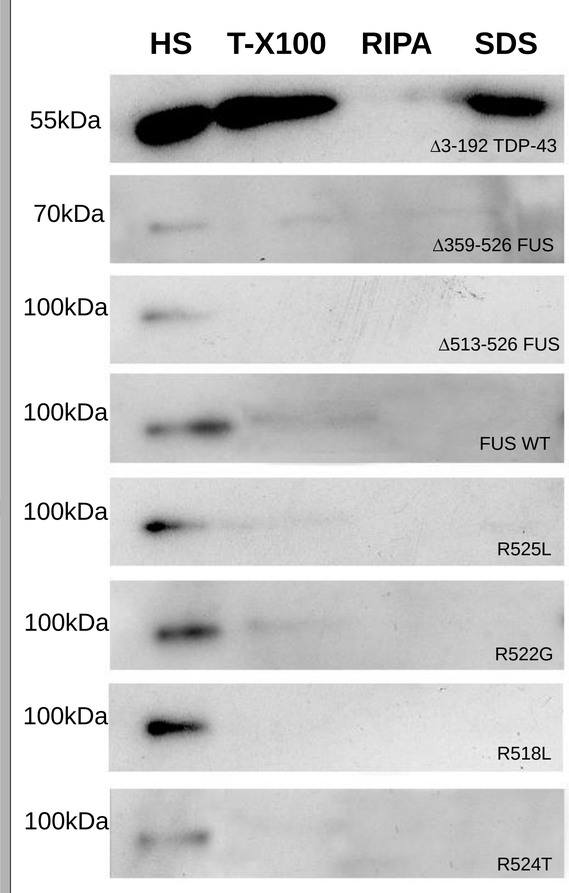


Figure 2 Fractionation and immunoblot analysis of transfected SH-SY5Y cells with EGFP-C1 vector with carrying the insertion of corresponding protein – FUS/TLS or TDP-43 – revealed detergent-soluble forms of FUS/TLS protein and detergent-soluble and insoluble forms of highly truncated TDP-43 protein.

HS – high salt buffer (soluble forms), T-X100 – HS buffer +Triton X-100, SDS – Laemmli buffer loading for SDS-PAGE. Living colors® (Invitrogen) antibodies were used for GFP-labelled protein detection.

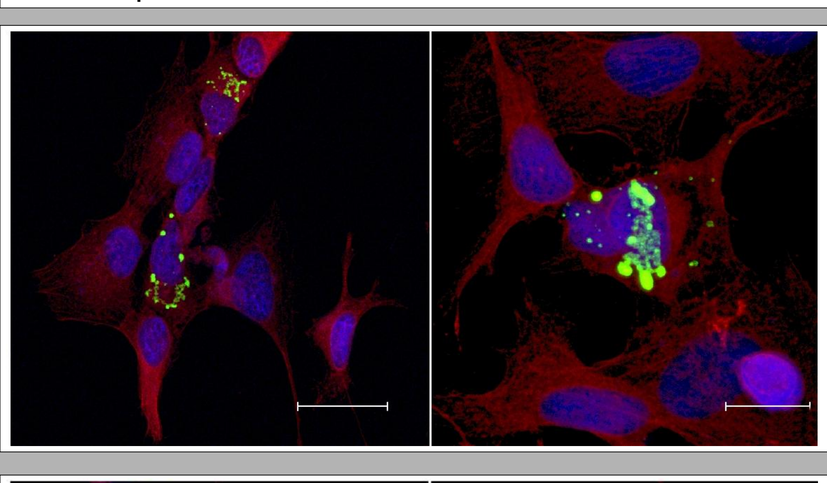


Figure 3 Highly truncated form of FUS/TLS (Δ359-526) lacking both nuclear localization and nuclear export signals as well as most of the RNA-recognition motif. Mutated protein is completely depleted from the nucleus and forms cytoplasmic aggregates immediately after synthesis (the aggregates could be detected in the cells even in 4 hours after transfection) indicating strong ability to aggregate for this form. The form was significantly more cytotoxic for cells compared to truncated TDP-43 Δ3-192.

Red - β-actin; green - FUS/TLS (Δ359-526)-GFP; blue - DAPI.

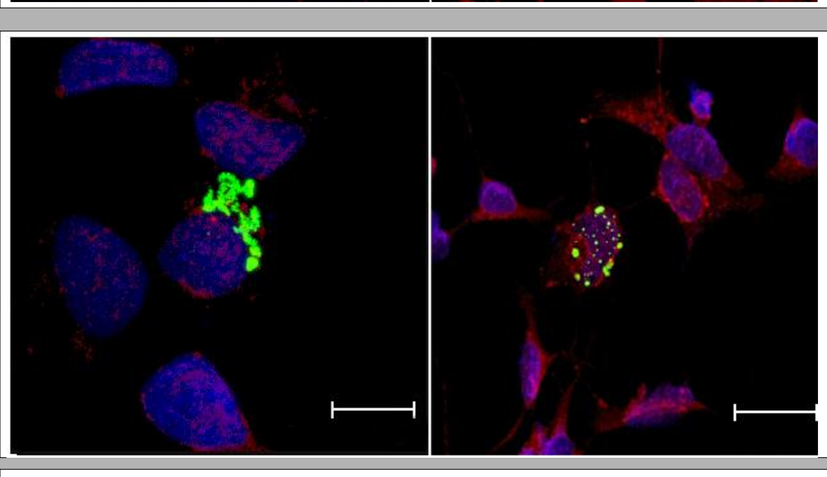


Figure 4 Anti-ubiquitin immunofluorescent labelling of SH-SY5Y transfected with highly truncated form of FUS/TLS (Δ359-526) and truncated form of TDP-43Δ-192 did not reveal ubiquitination neither cytoplasmic nor intranuclear inclusions.
This may be due to the fact that ubiquitination of TDP-43- or FUS-positive inclusions is a late event whereas transfected cells were fixed and immunolabeled within 24 h after transfection.

Red - β-actin; green - FUS/TLS (Δ359-526)-GFP or TDP-43 Δ3-192-GFP; blue - DAPI.

Conclusions

1. In the studied model system inclusions formed by pathological variants of FUS/TLS and TDP-43 are different, suggesting that they might have differently affects cell functions.

2. Our results confirm that as it has been predicted according to the sequence homology NLS in FUS/TLS includes residues 514-526. Loss of this sequence leads to protein depletion from the nucleus.

3. Localization studies showed that some of the mutations in NLS of FUS protein partially or completely abolish nuclear localization (i.e. R525L, R522G) while others do not (R524T, R518L). Similarly, compared to WT protein R524T, R518L and R522G do not affect mutant protein propensity to aggregate whereas R525L increased this ability. C-terminal truncation of FUS (Δ359-526) not only abolishes nuclear localization of FUS but also strongly increases mutant protein propensity to aggregate.

4. N-terminal truncated form of TDP-43 (Δ3-192) is able to form both nuclear and cytoplasmic inclusions which appear to be detergent-insoluble in fractionation studies.

5. Truncated Δ359-526 FUS/TLS is highly cytotoxic for cells.

6. Aggregates formed by both truncated forms, Δ359-526 FUS/TLS and Δ3-192 TDP-43, seem not to be ubiquitinated.

Results of our studies suggest that although pathological changes in TDP-43 and FUS/TLS structure and function lead to the same clinical manifestations, molecular and cellular mechanisms underlying the development of neurodegeneration might be different.

References

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