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 familial 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-SYSY human neuroblastoma cell lines expressing wild type or various mutated and truncated forms of FUS/TLS as eGFP fusion proteins.

Contraction of mutated forms in eGFP-C1 vector

Outline of the study

Toxicity studies

Immunocytochemical studies

Biochemical studies

SH-SYSY neuroblastoma cells

Number of cells with lost adhesion ability

Time point

Empty vector, cells per 0.1 µL ± SEM

FUS/TLS ∆359-526, 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

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) whereas 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.

4. N-terminal truncated form of FUS/TLS (∆3-192) is able to form both nuclear and cytoplasmic inclusions which appear to be detergent-insoluble in fractionation studies.

5. Truncated ∆359-526 FUS/TLS is highly cytotoxic for cells.

6. Aggregates formed by both truncated forms, ∆359-526 FUS/TLS and ∆359-526 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.

Figure 1 Distribution of TDP-43 and FUS/TLS and morphological types of aggregates formed by these two proteins in SH-SYSY neuroblastoma cells upon transfection with corresponding construct.

Green – protein+GFP; blue – DAPI.