ALS-associated p62 mutations alter TDP-43 solubility and localization in a cell culture model of disease

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor loss and has been linked to negatively affecting cognition and behavior due to neuronal loss in the frontotemporal cortex. There is a median survival time of only 2 to 3 years from the onset of symptoms with no current diagnostic test or cure for the disease.

The transactive response (TAR) DNA-binding protein 43 kDa (TDP-43) is a transcription factor and involved in RNA processing. Its abnormal aggregation and cytoplasmic localization has been identified as a pathological hallmark of disease.

A lack of a nuclear localization sequence (dNLS) in the TDP-43 gene can be expressed to mimic cytoplasmic localization as found in diseased patients. Further, TDP-43 aggregation can be mimicked using a single (KQ) or double (2KQ) mutation of a lysine residue to a glutamine residue.

Mutations in the sequestosome 1 (p62/SQSTM1) gene at points within various domain regions of the gene have been implicated in patients with disease. p62 is a scaffold protein involved in proteostasis, such as autophagy, degradation, and cell proliferation.

Therefore, this study investigated how different p62 mutants affect TDP-43 aggregation and localization.

MATERIALS AND METHODS

This study analyzed the effects of single mutations in p62 in various domain regions. HEK293 (human embryonic kidney) cells were transfected to co-express TDP variants and p62 mutants.

Western Blot: Cells were lysed and fractionated into soluble and insoluble fractions then immunoblotted.

Immunofluorescence: The TDP variants were GFP-tagged. Cells were cultured on glass coverslips then immunoblotted with fluorescent antibodies.

FIGURES AND RESULTS

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Domain Mutation
PB1 K102E, V90M
ZZ E155K
TBS K238del, P228L
LIR L341V, D337E
KIR P348L
UBA G411S

DISCUSSION AND CONCLUSION

The various p62 mutants co-expressed with the TDP-43 variants show average, increased, or decreased protein amounts as seen in both the western blots and the quantification of their signal intensities. In particular, the following mutants show the most abnormal protein expressions: K102E, V90M, E155K, K238del, L341V, G411S.

Taking this into account, the immunofluorescent microscopy shows abnormal localization to the cytoplasm for all but one mutant of the above variants. The only mutant p62 gene that did not seem to show cytoplasmic localization was G411S.

For future experiments, more mutants within these domains that show abnormal protein expression should be looked into to see which domains are more or less important in proteinopathy. Additionally, the experiments with immunofluorescence can be repeated, specifically with the G411S mutant along with other p62 mutants, in order to see if mutants causing abnormal aggregation concur with either nuclear or cytoplasmic localization.

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