THE FUS PROTEIN'S ROLE IN HUNTINGTON'S DISEASE

THE FUSS ABOUT FUS

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INTRODUCTION

Huntington disease is a genetic disorder that causes the progressive degeneration of nerve cells in the brain. Symptoms from Huntington disease include the loss of cognitive ability, mobility dysfunctions and psychiatric disorders. This disease is an autosomal dominant disorder which is expressed when the Huntingtin gene has a defect.

Protein aggregation is a biological phenomenon in which misfolded protein aggregates accumulate and clump together (either intra- or extracellularly). Aggregates in Huntington are found to contain many useful and important molecules which are inaccessible to the cell. Existing mechanisms adequately deal with most forms and degrees of misfolding. Nonetheless, destabilising mutations, extreme environments or ageing can overwhelm proteostasis leading to the unwanted accumulation of aggregated proteins which are too large to be "recycled" by the proteasome (a protein complex which breaks apart peptide bonds).

FUS is a gene which encodes a versatile protein found in the heterogeneous nuclear ribonucleoprotein (hnRNP) complex. FUS is responsible for cellular processes such as transcription regulation, RNA splicing, RNA transport and DNA repair. The FUS protein shuttles between the nucleus and cytoplasm. Defects in this gene result in ALS for two main reasons: either the FUS is incapable of performing its multifarious operations effectively, or the misfolded FUS forms aggregates in the nucleus which in turn has deleterious effects on the cells overall capability. The accumulation of nonfunctional FUS proteins increases the cells stress level which subsequently induces senescence. In addition to the association between FUS and ALS, FUS proteins have been found in Huntingtin aggregates suggesting a connection to Huntington disease as well.

LOCALISATION OF FUS TO HTTQ AGGREGATES

Figure (0)
The immunofluorescence of FUS and Huntingtin clearly show the formation of a FUS ring around the Huntingtin aggregates.

Produced by Jeeda Bishara

METHODOLOGY

TRANSFECTION

Transfection is the artificial introduction of foreign nucleic acids into cells. For the control, we inserted the HTT-17Q plasmid containing both the Huntingtin gene and GFP gene (a fluorescent gene) into HEK 293 cells. We repeated this process to a separate group of cells and in replace of the HTT-17Q we used HTT-134Q (the mutated Huntingtin). The succession of this process is shown in Figure 2.

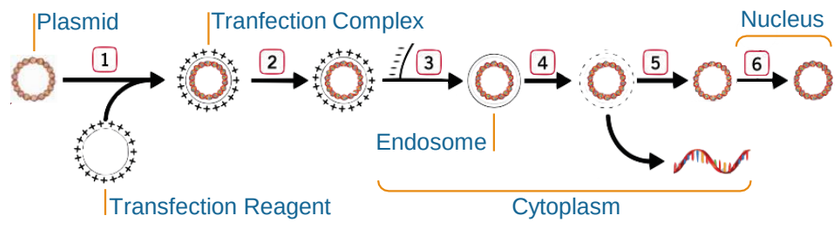


Illustration 1: A diagram of the different stages in the transfection method.

WESTERN BLOT

The Western Blot method is a technique which allows researchers to detect specific proteins extracted from cells.

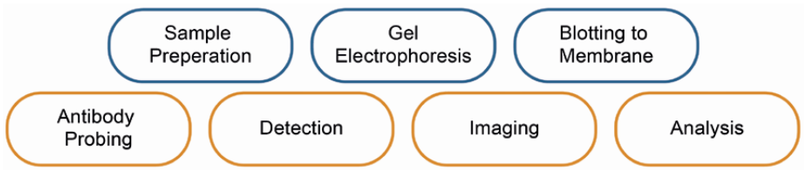


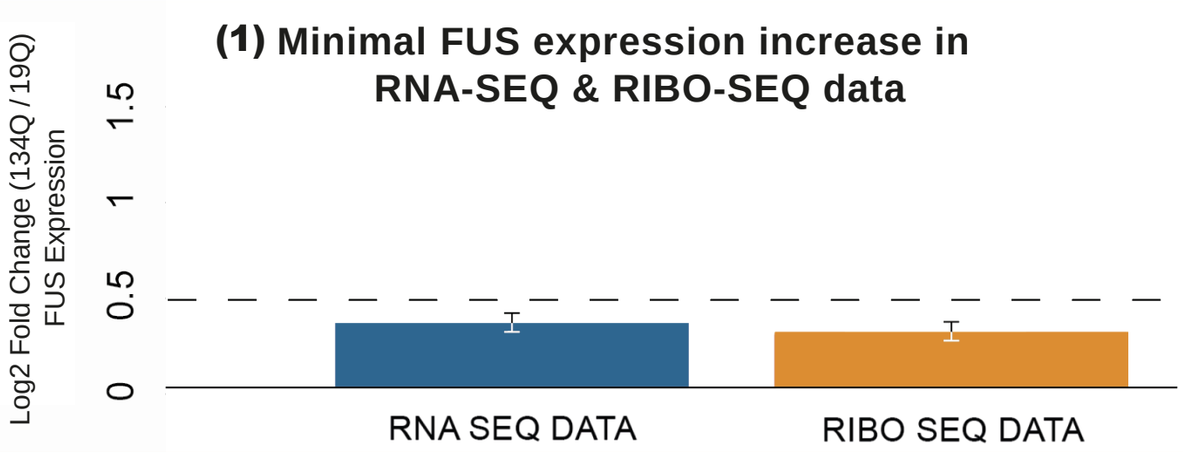
Illustration 2: A diagram of each stage in the Western Blot method.

RNA SEQ & RIBO SEQ ANALYSIS

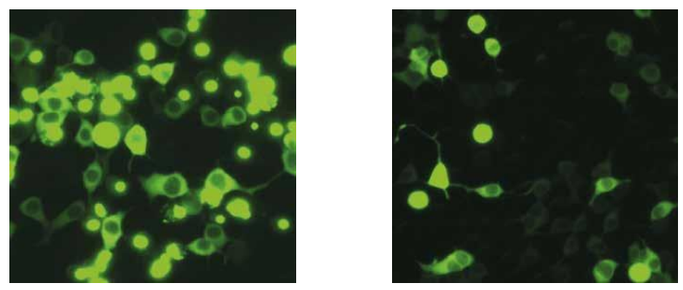
Analysis of RNA sequences and Ribosome profiling of cells transfected with HTT-134Q plasmids and HTT-19Q plasmids.

RESULTS

(1) Minimal FUS expression increase in RNA-SEQ & RIBO-SEQ data

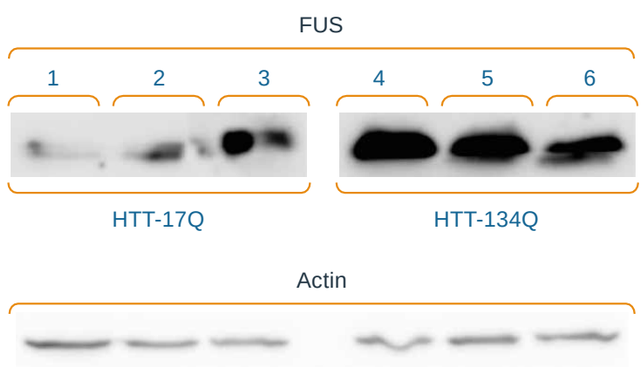


(2) Flourescent HTT Cells After Transfection

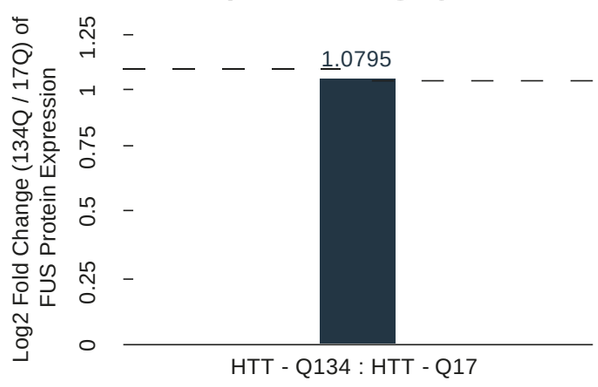


(3) Western Blot

(a) Chemiluminescent Membrane Photo



(b) FUS expression highly induced



(4) FUS Targets Compared to all Expressed Isoforms are Decreasing

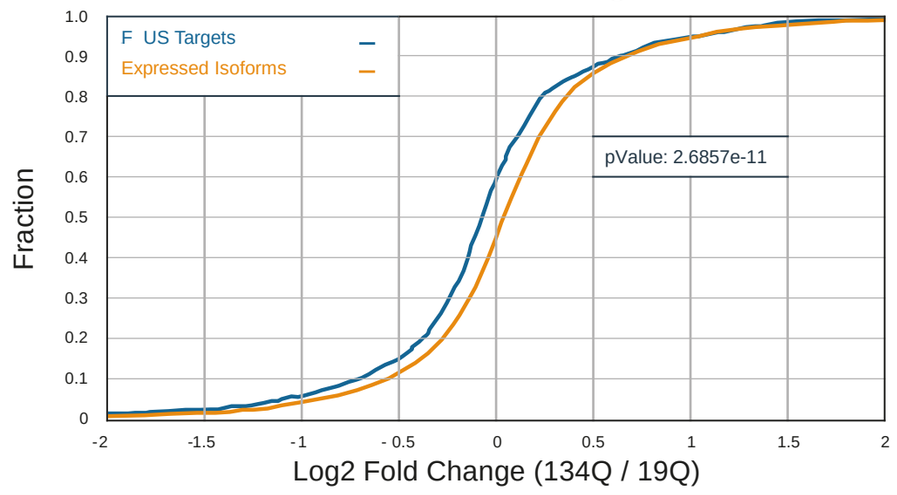


Figure (1)
The (blue) RNA-SEQ and the (orange) RIBO-SEQ diagram displays the difference between the amount of transcripted RNA, and the RNA translated by ribosomes. It appears that both expressions are mildly increasing (as their values are less than 0.5 LFC) which indicates the production change of FUS mRNA is insignificant.

Figure (2)
Both the HTT-17Q and HTT-134Q plasmids contain the Huntingtin gene and GFP gene. The concentration of green dots in mHTT-139Q represent the clusters of Huntingtin (the aggregates).

Figure (3a & 3b)
Using the Western Blot membrane from Figure 3a an accurate reading of each band enabled us to calculate the FUS protein levels. For each lane, a ratio was calculated between the HTT and Actin to normalise the data. An average intensity was calculated for both the Huntington and mutant Huntingtin gene. Finally, we performed the log2 Fold Change calculation and saw a protein level increase in the mutant cells shown in Figure 3b.

Figure (4)
It is evident that the FUS Targets [1] are shifted to the left of the Expressed Isoforms, specifically from -1.5 to 0.6 on the Log2 Fold Change axis. This clearly shows the decreased expression of RNA within the mutant cells of FUS Targets.

Conclusions

To conclude, the results we examined demonstrate how FUS is being clustered together inside the mHTT as opposed to the controlled HTT. According to Figure 1, it is evident that FUS production is barely increasing, on the other hand, its protein levels are significantly greater [Figure 3a & 3b]. Thus, we can deduce that the degradation/removal of the FUS protein is decreasing. To speculate, we believe the FUS is being trapped within the mHTT aggregates which prevent the proteasome from dismantling the FUS. Our speculation is justified as FUS is shown to have a lack of functionality in Figure 4 where the mutant Huntingtin is present.

Acknowledgements

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References

[1] Hoell, Jessica I., et al. "RNA targets of wild-type and mutant FET family proteins." Nature structural & molecular biology 18.12 (2011): 1428.