HuD regulation of SOD1 and FUS mRNAs in sporadic ALS

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Objectives: Neuronal ELAV RNA-binding protein (RBP) HuD has been previously associated with neurodegenerative diseases (NDs) [1], [2]. Bioinformatics analysis of SOD1 and FUS 3'UTRs demonstrated the presence of HuD consensus binding sequences in these mRNAs.
We aimed to test whether HuD levels are altered in ALS and how this affects levels and localization target mRNAs.

Methods. Using human neuroblastoma SH-SY5Y cells as an *in vitro* model of ALS pathophysiology and *post-mortem* tissues from sporadic ALS patients we evaluate HuD and its targets level by qRT-PCR, WB and Immunofluorescence (IF) analyses. Through RNA immunoprecipitation (RIP) assays we tested HuD binding on SOD1 and FUS 3'UTR.

Results. The induction of a neuronal-like phenotype triggers a significant increase in HuD mRNA levels and an oxidative stress-dependent overexpression of SOD1 and FUS mRNAs.

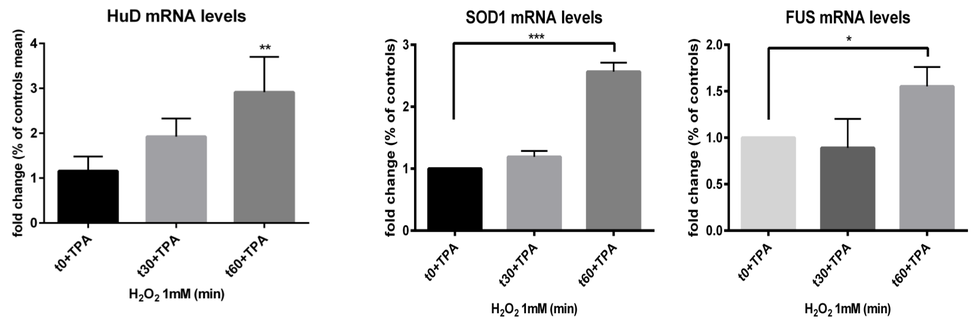
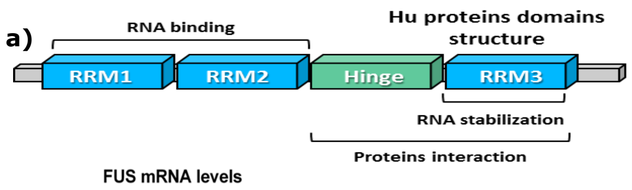


Figure 1 Determination of HuD, SOD1 and FUS mRNA levels in human neuroblastoma SH-SY5Y cells differentiated with 160 nM TPA and treated with 1 mM H_2O_2 for 30, and 60 min.

The increase in target mRNA is likely due to the stabilization, as demonstrated by the significant reduction of SOD1 and FUS mRNAs after the overexpression of HuD mutant protein lacking the RNA Recognition Motif 3 (RRM3).



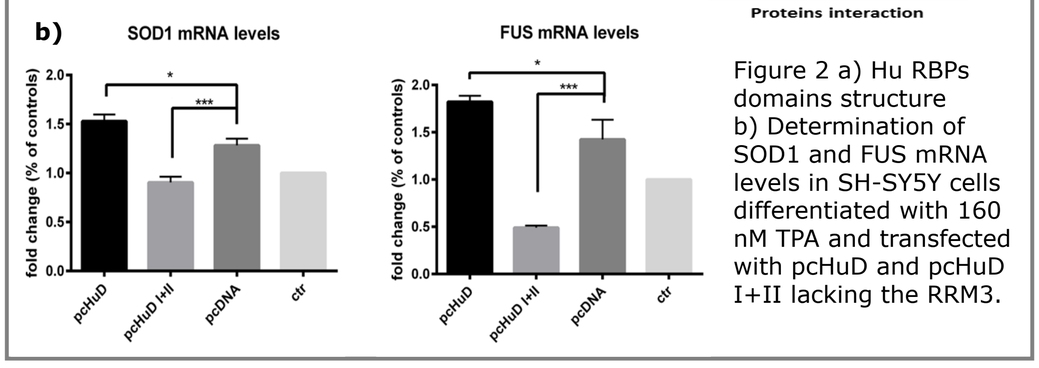


Figure 2 a) Hu RBPs domains structure
b) Determination of SOD1 and FUS mRNA levels in SH-SY5Y cells differentiated with 160 nM TPA and transfected with pcHuD and pcHuD I+II lacking the RRM3.

By RIP we demonstrated that HuD binding to SOD1 mRNA is oxidative stress dependent while FUS mRNA is more likely regulated by HuR.

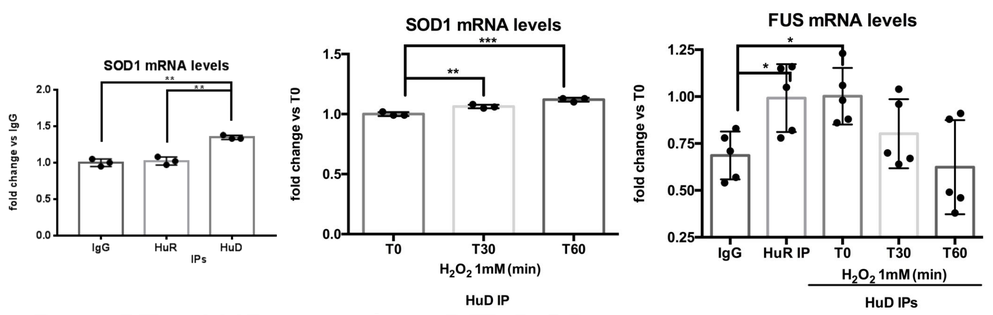
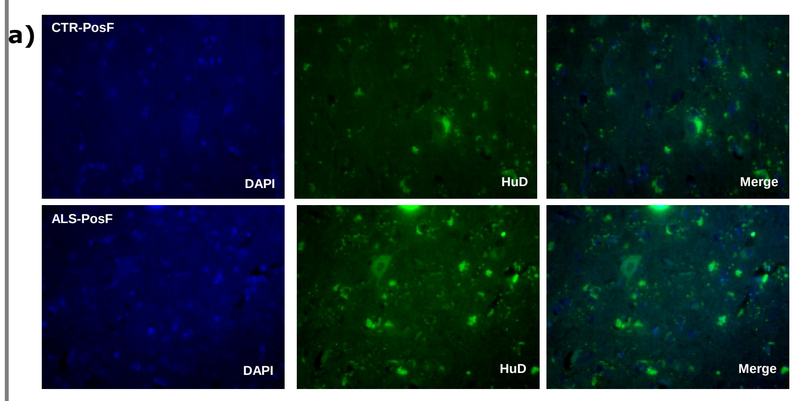
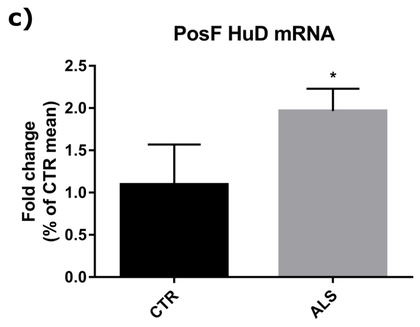


Figure 3 Validation of HuD binding to SOD1 and FUS 3'UTR in human neuroblastoma SH-SY5Y cells differentiated with 160 nM TPA and treated with 1 mM H_2O_2 for 30, and 60 min.

By IF, WB and qRT-PCR experiments in post-mortem tissues from sporadic ALS patients we found that HuD protein levels were increased in the Posterior frontal cortex (PosF) compared to healthy controls.





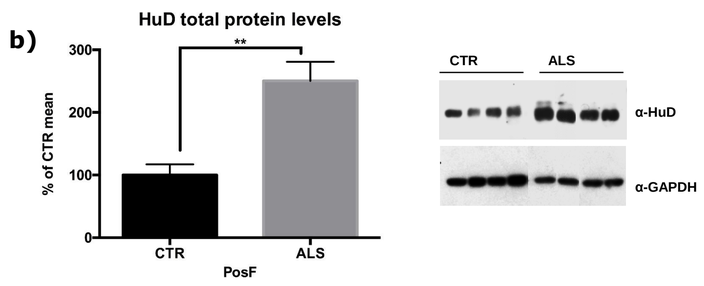
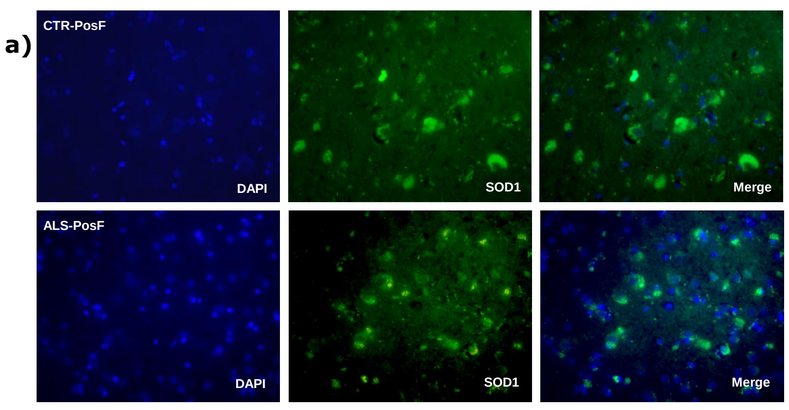
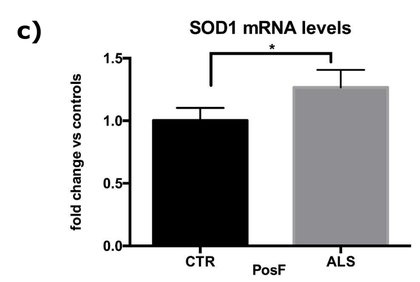
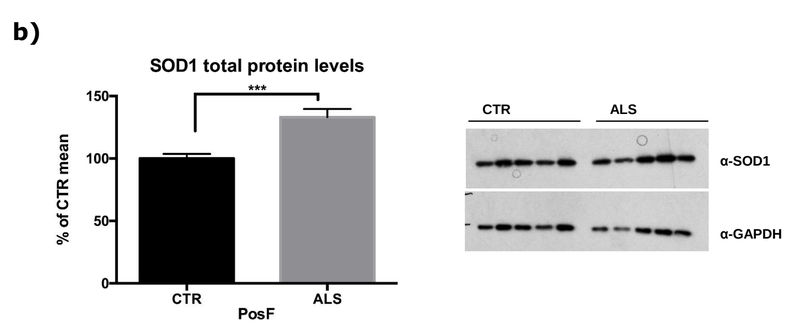


Figure 4 a-b) Determination of HuD protein levels in PosF of ALS patients and controls by IF analysis of HuD (green) and WB of total proteins extract.
c) qRT-PCR evaluation of HuD mRNA levels in PosF of ALS patients and controls.

We also found increased SOD1 protein levels in the PosF from ALS patients along with increases in mRNA due to HuD binding on its 3'UTR.







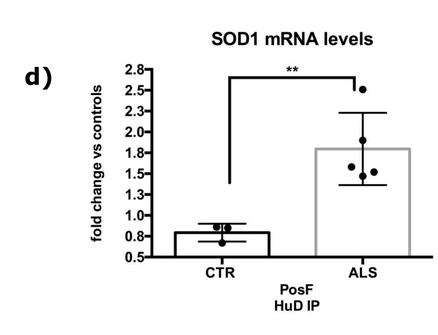


Figure 5 a-b) Determination of SOD1 protein levels in PosF of ALS patients and controls by IF analysis of SOD1 (green) and WB of total proteins extract. c) qRT-PCR evaluation of SOD1 mRNA levels in PosF of ALS patients and controls. d) HuD binding to SOD1 3' UTR by RIP assay in PosF of ALS patients and controls.

Conclusions: Uncovering HuD post-transcriptional regulation of SOD1 and FUS mRNAs will open novel perspectives for ALS research and the identification of new therapeutic targets.

[1] P. Milani, M. et al., "Posttranscriptional regulation of SOD1 gene expression under oxidative stress: Potential role of ELAV proteins in sporadic ALS," *Neurobiol. Dis.*, vol. 60, pp. 51–60, 2013.

[2] L. Lu, et al., "Hu Antigen R (HuR) Is a Positive Regulator of the RNA-binding Proteins TDP-43 and FUS/TLS: IMPLICATIONS FOR AMYOTROPHIC LATERAL SCLEROSIS," *J. Biol. Chem.*, vol. 289, no. 46, pp. 31792–31804, 2014.

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