HuR stabilizes *HTT* mRNA via interacting with its exon 11 in a mutant HTT-dependent manner

Chen Li[1], Quan Zhao[1], Xiaoli Sun[1], Boxun Lu[1,†]
1 School of Life Sciences, Fudan University, Shanghai, China.

Abstract

Huntington's Disease (HD) is a monogenetic neurodegenerative disorder mainly caused by the cytotoxicity of the mutant HTT protein (mHTT) encoded by the mutant *HTT* gene. Lowering *HTT* mRNA has been extensively studied as a potential therapeutic strategy, but how its level is regulated endogenously has been unclear. Here we report that the RNA-binding protein (RBP) HuR interacts with and stabilizes *HTT* mRNA in an mHTT-dependent manner. In HD cells but not wild-type cells, siRNA knockdown or CRISPR-induced heterozygous knockout of HuR decreased *HTT* mRNA stability. HuR interacted with *HTT* mRNA at a conserved site in exon 11 rather than the 3'-UTR region of the mRNA. Interestingly, this interaction was dependent on the presence of mHTT, likely *via* the activation of MAPK11, which enhanced cytosolic localization of the HuR protein. Thus, mHTT, MAPK11 and HuR may form a positive feedback loop that stabilizes *HTT* mRNA and enhances mHTT accumulation, which may contribute to HD progression. Our study reveals a novel regulatory mechanism of *HTT* mRNA *via* non-canonical binding of HuR.

Background

Huntington's disease is a monogenetic neurodegenerative disorder mainly caused by the mHTT cytotoxicity. Recently, gene therapy approaches targeting *HTT* mRNA have obtained tremendous success in developing potential treatments for HD mammalian models. Meanwhile, how *HTT* mRNA is regulated endogenously has been less well understood. This mechanism may worth studying because it may provide novel insights into HTT biology and potential therapeutic targets for HD. We have previously demonstrated MAPK11 as a novel kinase modulator of *HTT* mRNA stability, giving us an entry point to identify potential RBPs regulating *HTT* mRNAs.

Methods

1. Cell culture
2. cDNA and siRNA transfection
3. RNA immunoprecipitation (RNA-IP)
4. RT-qPCR and mRNA stability measurements
5. RNA electrophoretic mobility shift assay (R-EMSA)
6. Nuclear/cytoplasmic protein extraction and Western-blots
7. Immunofluorescence and high-content imaging

Results

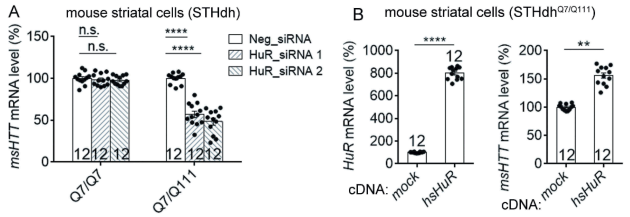

Fig. 1 | HuR modulates *HTT* mRNA levels in an mHTT dependent manner. RT-qPCR measurements of *HuR* and *HTT* mRNA level in wild-type (STHdhQ7/Q7) or HD (STHdhQ7/Q111) mouse striatal cells transfected with the siRNA targeting HuR and non-targeting control siRNA for 48 h (A), or transfected with the hsHuR cDNA (B).

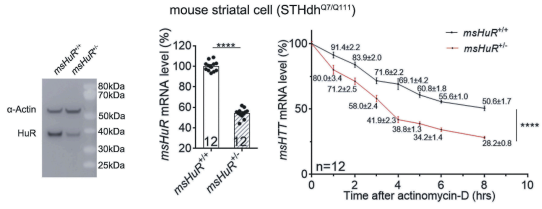

Fig. 2 | Lowering HuR by siRNA knockdown or CRISPR heterozygous knockout decreased *HTT* mRNA stability in HD cells. *Left* panel: Western-blot of HuR in HD cells with or without *HuR* knocked out by the CRISPR system. *Right* panel: HTT mRNA stability measurements in HD cells.

Acknowledgement

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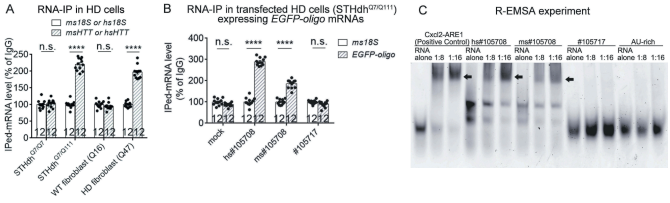

Fig. 3 | HuR interacts with #105708 site in exon 11 of the mutant *HTT* mRNA. RT-qPCR quantifications of HuR-bound endogenous HTT by RNA-IP in HD cells (A), or exogenous expressed EGFP-oligo mRNAs containing candidate binding sites (B). EMSA of Cy3-labelled RNA oligos of potential binding sequences after co-incubation with HuR-MBP (C).

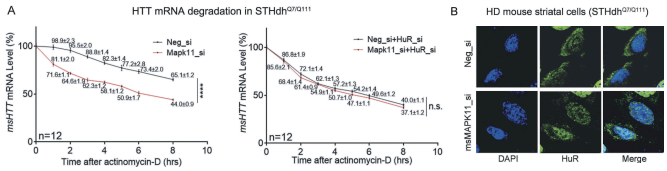

Fig. 4 | MAPK11 is an upstream modulator of HuR and controls HuR's regulation of *HTT* mRNA levels in HD cells. (A) *HTT* mRNA degradation measurements in HD cells transfected with the indicated siRNAs for 36 h, then treated with actinomycin D. (B) Immunofluorescent staining of the distribution of HuR protein in nuclear and cytoplasm after treated with indicated siRNA.

Conclusion

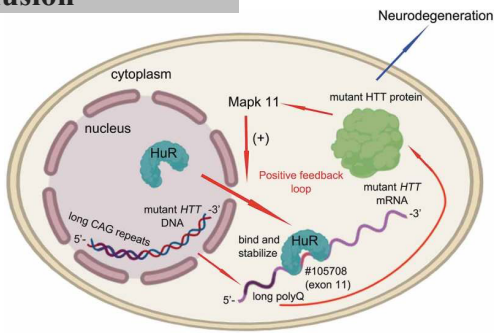

Fig. 5 | A schematic model illustrating mHTT-dependent MAPK11-HuR mediated regulation of *HTT* mRNA stability. We identified HuR as the RNA binding protein that interacts with *HTT* mRNA and stabilizes it, in a mutant HTT dependent manner. Upstream regulator MAPK11 modulates HuR and regulated nuclei/cyto-plasm distribution of HuR protein in HD cells or tissues.

Key References

[1] Yu M, et al. Suppression of MAPK11 or HIPK3 reduces mutant Huntingtin levels in Huntington's disease models. *Cell Res.* 2017; 27:1441–1465

[2] Zhao Q, et al. HuR stabilizes *HTT* mRNA *via* interacting with its exon 11 in a mutant HTT-dependent manner, *RNA Bio*. 2020; 17(4): 500-516