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

Quan Zhao, Chen Li, Meng Yu, Yimin Sun, Jian Wang, Lixiang Ma, Xiaoli Sun, and Boxun Lu

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 data reveal a novel regulatory mechanism of HTT mRNA via non-canonical binding of HuR.

Introduction
Huntington’s disease (HD) is an important monogenetic neurodegenerative disorder mainly caused by the mutation of the HTT gene [1], which encodes the mutant HTT protein (mHTT) with expanded polyglutamine tract (polyQ) [2,3], mHTT cytotoxicity is the major cause of HD, while the underlying molecular mechanisms remain unclear and likely involve multiple pathways [2]. Recently, gene therapy approaches targeting HTT mRNA have obtained tremendous success in developing potential treatment for HD: in several mammalian models, delivery of short hairpin RNAs (shRNAs) or small interference RNAs (siRNAs) or antisense oligonucleotides (ASOs) targeting HTT mRNA lowered HTT protein levels and attenuated neuropathology and disease-related phenotypes [4–7]; More importantly, an ASO targeting HTT mRNA, HTT-Rx developed by IONIS, has been investigated in clinical studies and obtained preliminary success in Phase I/IIa clinical trial [8]. 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.

The stability of HTT mRNA could be influenced by non-coding RNAs including an anti-sense LncRNA expressed by the reverse strand of the HTT gene [9]. On the other hand, RNA-binding proteins (RBPs) have been shown to modulate the stability of many mRNAs by interacting with them, playing key roles in many neurodegenerative disorders such as ALS/FTD [10]. Thus, whether and which RBPs regulate HTT mRNA is of interest in the HD and the RNA stability research field and yet remains unknown.

We have previously demonstrated MAPK11 as a novel kinase modulator of HTT mRNA stability [11], giving us an entry point to identify potential RBPs regulating HTT mRNAs. In this study, we identified HuR as the RBP that interacts with HTT mRNA and stabilizes it. We further elucidated relevant molecular mechanisms and the binding site, providing novel insights into HTT mRNA regulation and new pathobiology function of HuR.

Results
The RBP HuR is a potential modifier of HTT mRNA levels in HD cells in an mHTT-dependent manner
Our previous studies have shown that MAPK11 modulates the stability of HTT mRNA. To identify the potential downstream RBP(s) that interact with HTT mRNA, we investigated candidate RBPs that may interact with HTT mRNA based on the CLIP-seq data analysed by StarBase (http://starbase.sysu.edu.cn/) [12] (Fig. 1A). We then checked the brain expression of this panel of candidate RBPs based BioGPS (http://biogps.org) [13] and

CONTACT
Boxun Lu, luboxun@fudan.edu.cn Neurology Department at Huashan Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Institutes of Biomedical Sciences, School of Life Sciences, Fudan University, Shanghai, China; Department of Anatomy, Histology and Embryology, Shanghai Basic Medical College, Fudan University, Shanghai, China; Shanghai Xuhui District Central Hospital, Zhongshan Xuhui Hospital, Fudan University, Shanghai, China; Shanghai Xuhui District Central Hospital, Zhongshan Xuhui Hospital, Fudan University, Shanghai, China

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group. This is an Open Access article published under the terms of the Creative Commons Attribution-NoCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.
selected those brain-expressing RBPs with more than 5 potential HTT mRNA targeting sites for further testing (Fig. 1A). We then investigated their potential influences on mHTT levels in immortalized HD patient fibroblasts (Q45 and Q68) by the well-established HTRF (homogeneous time-resolved fluorescence) assay using the 2B7/MW1 antibody pair. Transfection of pooled siRNA targeting ELAVL1 (referred to as ‘HuR’ in the subsequent text to keep consistency with the literature), but not the other RBP expressing genes significantly lowered mHTT levels in both Q45 and Q68 patient fibroblasts (Fig. 1B), revealing HuR as a candidate RBP that regulates HTT mRNA.

To confirm HuR as an HTT mRNA regulator, we tested the effect of HuR knockdown in HD cells including the HD knockin mouse striatal cells STHdh<sup>Q7/Q111</sup> [14] and immortalized human HD patient fibroblasts (Q45 & Q68) by the well-established HTRF (homogeneous time-resolved fluorescence) assay using the 2B7/MW1 antibody pair. Transfection of pooled siRNA targeting ELAVL1 (referred to as ‘HuR’ in the subsequent text to keep consistency with the literature), but not the other RBP expressing genes significantly lowered mHTT levels in both Q45 and Q68 patient fibroblasts (Fig. 1B), revealing HuR as a candidate RBP that regulates HTT mRNA.

HuR positively regulates HTT mRNA stability

As a member of the ELAVL family RBPs, HuR may stabilize target mRNAs by its direct interaction with them [15]. Thus,
Figure 2. HuR modulates HTT mRNA levels in a mHTT dependent manner.

(A) RT-qPCR measurements of HuR and HTT mRNA level in wild-type (STHdh<sup>Q7/Q7</sup>) or HD (STHdh<sup>Q7/Q111</sup>) mouse striatal cells transfected with the siRNAs targeting HuR (HuR<sub>siRNA</sub> 1 and HuR<sub>siRNA</sub> 2) or with the non-targeting control siRNA (Neg<sub>siRNA</sub>) for 48 hours (12 technical replicates from 3 biological replicates). All signals were normalized to the averaged signal of Neg<sub>siRNA</sub> transfected controls.

(B) Similar to (A), but in immortalized human wild-type (Q16) or HD (Q47) fibroblasts (12 technical replicates from 3 biological replicates).

(C) HTRF measurements of HTT protein levels in the HuR siRNA or the non-targeting control siRNA (Neg<sub>siRNA</sub>) transfected wild-type or HD cells (12 technical replicates from 3 biological replicates for STHdh cells, and 16 technical replicates from 4 biological replicates for immortalized human fibroblasts). The siRNAs were transfected for 72 hours. All signals were normalized to the Neg<sub>siRNA</sub> transfected controls. Note that the HTRF antibody pair 2B7/2166 was used here to detect the total HTT (including mHTT and wild-type HTT proteins) levels, different from the mHTT-specific antibody pair used for Fig. 1B.

(D) RT-qPCR measurements of HuR and mouse HTT (msHTT) mRNA levels in the HD mouse striatal cells (STHdh<sup>Q7/Q111</sup>) transfected with cDNA plasmids expressing human HuR. All signals were normalized to the averaged signal of the empty vector transfected control group (mock) (12 technical replicates from 3 biological replicates). Note that the HuR mRNA measurement in the mock group utilized the mouse HuR qPCR primers, whereas the one in the HuR transfected group utilized human HuR qPCR primers. Since these two set of primers had similar efficiency (Fig. S5) and the same threshold was used for analysis, the comparison between these two groups is valid.

(E) RT-qPCR measurements of HuR and mouse HTT (msHTT) mRNA level in the wild-type mouse striatal cells (STHdh<sup>Q7/Q7</sup>). The cells were transfected with the siRNAs targeting HuR or the non-targeting control siRNA (Neg<sub>siRNA</sub>) and then after 24 hours with cDNA plasmids expressing human wild-type full-length HTT (hsHTT-Q23), mutant HTT exon1 (hsHTT<sub>exon1</sub>-Q72) or full-length (hsHTT-Q73) (12 technical replicates from 3 biological replicates). The mRNA levels were then measured 48 hours after cDNA transfection. All the signals were normalized to the averaged signals of the Neg<sub>siRNA</sub> transfected control groups. For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by one-way ANOVA and post-hoc Dunnett's tests for A-C, and two-tailed unpaired t tests for D-E. **P < 0.01; ****P < 0.0001; n.s., P > 0.05.
the most likely mechanism of HuR-mediated regulation of 
HTT mRNA levels is via RNA-stabilization. We thus exam-
ined HTT mRNA degradation in the HD cells by measuring 
the levels of mRNAs after treatment of actinomycin D, which 
stops new RNA synthesis by inhibiting RNA polymerase 
activity so that the degradation of pre-existing mRNAs 
could be measured by qPCR directly. In HD cells, including 
STHdhQ7/Q111 and immortalized human patient fibroblasts 
(Q47), transfection of HuR siRNAs significantly increased 
the degradation of HuR mRNA via RNA interference 
(Fig. 3A, B, left panels). Importantly, both msHTT and 
hsHTT mRNAs were also degraded significantly faster in 
HuR knockdown cells than in the control cells, suggesting 
that HuR is required for HTT mRNA stabilization in these 
cells (Fig. 3A, B, right panels).

To further confirm this conclusion, we attempted to knock 
out the HuR gene in STHdhQ7/Q111 cells by the CRISPR/cas9 
system, but only managed to obtain pooled colonies of het-
ierozygous HuR knockout cells (HuR+, Fig. 3C), possibly 
because a complete loss of HuR is cytotoxic. The HTT 
mRNA degradation in the HuR+ STHdhQ7/Q111 cells was 
also significantly faster than in the HuR+/− STHdhQ7/Q111 
cells (Fig. 3C), consistent with the knockdown experiments. 
Taken together, HuR positively regulates HTT mRNA levels 
via increasing its stability.

The HuR protein interacts with HTT mRNA in its exon 11 
in the coding region

As an RBP, HuR may directly interact with HTT mRNA to 
stabilize it. In fact, previous PAR-CLIP-Seq data analysed in 
Starbase [12] suggested 25 potential HuR-binding sites on 
human HTT mRNA (Fig. 4A). To confirm the binding 
between HuR and the HTT mRNA, we performed RNA-
immunoprecipitation (RNA-IP) experiments in 
STHdhQ7/Q111 and immortalized Q47 fibroblasts. The HuR 
protein was pulled down by anti-HuR conjugated magnetic 
beads, and the amount of HTT mRNA that was pulled down 
with the HuR protein was remarkably higher than the control 
(pulled down by IgG-conjugated beads) (Fig. 4B), suggesting 
that the HuR protein interacts with HTT mRNA in these HD 
cells.

We further explored the HuR-binding site(s) in the HTT 
mRNA. Based on the analysis of Starbase (Fig. 4A), some of 
the potential binding sites are in the coding region, whereas 
some are in the 3’UTR. Thus, we exogenously expressed 
hsHTT cDNA with only the coding sequence but not the 
3’UTR sequence to test if at least some of the binding site(s) 
are within the coding region. We transfected the wild-type 
mouse STHdhQ7/Q7 cells with the plasmids that exogenously 
express human mutant HTT with full-length coding sequences 
(CDS) (hsHTT-Q73) or with exon 1 only (hsHTT exon1-Q72), 
and performed RNA-IP experiments for the exogenously 
expressed hsHTT mRNAs (not the endogenous msHTT 
mRNA) to test whether the HuR protein can bind with them. 
The empty vector transfected control sample (mock) did not 
give any hsHTT signals (Fig. 4C), confirming that the RNA-IP 
experiments and qPCR measurements only detect the binding to 
exogenously expressed hsHTT mRNAs. The HuR antibody- and 
IgG-conjugated beads pulled down similar levels of the 
hsHTT exon1-Q72 mRNA (Fig. 4C), suggesting that the binding 
site(s) are probably not within the exon 1 region. In comparison, 
the HuR was able to bind with the mutant full-length hsHTT 
CDS (Fig. 4C, hsHTT-Q73), suggesting that at least some of the 
binding site(s) are within the coding region. Interestingly, no 
binding with the wild-type full-length hsHTT CDS (Fig. 4C, 
hsHTT-Q23) was detected, suggesting that the binding is 
dependent on the presence of mHTT, consistent with previous func-
tional experiments (Fig. 2E). To further test if this binding is 
functionally relevant, we knocked down HuR mRNA in these 
cells and tested if these exogenously expressed hsHTT mRNA 
levels were influenced. Consistent with the RNA-IP data, only 
the levels of full-length mutant HTT CDS mRNA (hsHTT-Q73) 
were significantly reduced by HuR knockdown, but not the ones 
of its exon 1 fragment mRNA (hsHTT exon1-Q72) or the wild-
type full-length HTT CDS (hsHTT-Q23) (Fig. 4D). Collectively, 
our data suggest that HuR functionally interacts with the HTT 
mRNA in its protein coding region, but not in its exon 1.

To map the precise binding site(s), we reviewed all the 
potential binding sites based on StarBase (Fig. 4A) and blasted 
these sequences with the msHTT mRNA to see whether these 
sites are conserved between human and mouse, because HuR 
was able to interact with both hsHTT and msHTT to regulate 
their stability (Figs. 2 & 3). We found that only one candidate 
site, #105708, has conserved sequences between human and 
mouse (Fig. 4A, highlighted). Thus, #105708 is likely a HuR-
binding site that mediates HuR’s regulation of HTT mRNA 
stability. Since #105708 locates in exon 11 of the HTT mRNA, 
we tested if exon 11 is required for the interaction between 
HuR and the HTT mRNA. Thus, we constructed two plasmids 
expressing the first 10 (exon 1–10) or 11 exons (exon 1–11) 
of the mutant human HTT mRNA (hsHTT-Q73), respectively.

The only difference between these two plasmids is the pre-
ence of exon 11. We then transfected the wild-type mouse 
STHdhQ7/Q7 cells with these plasmids to test whether HuR 
could bind to the mRNAs expressed by these two plasmids. 
RNA-IP experiment results showed that the HuR protein 
interacted with exon 1–11, but not exon 1–10 (Fig. 5A), 
suggesting that the binding site is within the exon 11 region 
and likely to be #105708.

To confirm this, we cloned several candidate sequences 
(including #105708) into the EGFP construct, so that the 3’ 
UTR of EGFP mRNA contains these sequences. We then trans-
fected the HD mouse STHdhQ7/Q111 cells with these plasmids 
and tested whether HuR may interact with the EGFP mRNAs 
expressed from these plasmids. RNA-IP experiments showed 
that the EGFP mRNAs with #105708 sequences (hs#105708 
and ms#105708 for human and mouse sequences, respectively) 
but not the #105717 sequence exhibited interaction with HuR 
(Fig. 5B). The experiment also suggests that #105708 is sufficient 
for HuR binding in the HD cells.

To further validate #105708 as the binding site, we performed 
RNA electrophoretic mobility shift assays (R-EMSA) experiments 
to test the direct interaction between HuR and #105708. We 
mixed Cy3-labelled RNA oligos of either the human #105708 
(hs#105708) or the mouse #105708 (ms#105708) sequences with 
the HuR-MBP.His protein (with the human HuR amino acid 
sequence) purified from HEK293T cells (Fig. S3) at indicated
Figure 3. Lowering HuR by siRNA knockdown or CRISPR heterozygous knockout decreased HTT mRNA stability in HD cells.

(A) HuR mRNA (left) and HTT mRNA (right) stability measurements in HD mouse striatal cells (STHdh\(^{Q7/Q111}\)) (12 technical replicates from 3 biological replicates). Cells were transfected with indicated siRNAs for 36 hours, then treated with actinomycin D to stop new mRNA synthesis. HuR and HTT mRNA levels were then measured at different time points as indicated on the X-axis. The levels were then normalized to ms18S mRNA levels and plotted as the percentage of the time 0. Data values (mean ± S.E.M.) have been indicated for each point.

(B) Similar to (A), but in immortalized HD patient fibroblasts (Q47) (12 technical replicates from 3 biological replicates). Data values (mean ± S.E.M.) have been indicated for each point.

(C) Left panel: A representative Western-blot of HuR in HD mouse striatal cells (STHdh\(^{Q7/Q111}\)) with or without HuR knocked out by the CRISPR system. α-Actin was blotted as a loading control. The sequencing chromatograms also verified the heterozygous knockout of HuR from the population (lower panel). Right panel: similar to A, but in HuR\(^{−/−}\) or HuR\(^{+/−}\) STHdhQ7/Q111 cells without siRNA transfections (12 technical replicates from 3 biological replicates). Data values (mean ± S.E.M.) have been indicated for each point. For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by two-way ANOVA, except the middle panel of C, which was analysed by two-tailed unpaired t tests. ***P < 0.001; ****P < 0.0001.
ratios and ran the samples on native-PAGE gels (Fig. 5C). We observed apparent molecular weight-shift of these RNA oligos due to mobility block caused by HuR binding (Fig. 5C), suggesting that HuR interacts with both mouse and human #105708 sequences directly, consistent with the RNA-IP results (Fig. 5B).

As controls, we performed R-EMSA for an RNA oligo with known HuR-interacting sequence (Fig. 5C, Cxcl 2) [16], and observed expected apparent molecular weight shift as well. In addition, we tested two other candidate binding sites using R-EMSA. #105717 is a candidate binding site in the 3’UTR region (Fig. 4A). The ‘AU-rich’ oligo contains the only AU-rich element (ARE) in the HTT mRNA. Since AREs are the HuR preferred binding sites [15], we anticipated that the ‘AU-rich’ oligo might interact with HuR as well. Surprisingly, we observed no apparent molecular weight shift of RNA oligos with either of these two sequences (Fig. 5C), suggesting that #105708 is a relatively specific site in the HTT mRNA for HuR binding.

To investigate whether the HuR-#105708 interaction is functional in regulating mRNA levels in the cells, we cloned candidate binding site sequences into a firefly-renilla dual-luciferase reporter system and measured the luciferase activity in HD cells (Fig. 4B, C, D). We found that the HuR-#105708 interaction significantly increased the expression of the HTT mRNA in HD cells, suggesting that HuR may play a role in regulating the expression of HTT mRNA in HD cells.

Figure 4. HuR interacts with HTT mRNA in the HD cells, likely in the protein-coding region of HTT.

(A) A list of potential HuR’s binding sites in human HTT (hsHTT) mRNA based on StarBase (ref. [12]). The highlighted lines indicate a candidate site (#105708) that has conserved sequences in human and mouse. ‘AU-rich’ indicates a site that is not listed in StarBase, but is still a possible interaction site predicted by HuR preferred binding sequences [ARE]. (B) RT-qPCR quantifications of HuR-bound HTT mRNA levels in HD mouse striatal cells (STHdhQ7/Q111) or immortalized HD patient fibroblasts (Q47) as well as the wild-type controls (STHdhQ7/Q7 and Q16) by RNA-IP (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. (C) RT-qPCR quantifications of HuR-bound exogenously expressed human HTT (hsHTT) mRNA levels in WT mouse striatal cells (STHdhQ7/Q7) transfected with cDNA plasmids expressing full-length wild-type human HTT (hsHTT-Q23), full-length mutant HTT (hsHTT-Q73) or exon1 of mutant HTT (hsHTTexon1-Q72) by RNA-IP (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. (D) RT-qPCR quantifications of the exogenously expressed human HTT mRNA level in wild-type mouse striatal cells (STHdhQ7/Q7) transfected with the HuR siRNA or the non-targeting control siRNA (Neg_siRNA). The cDNA plasmids expressing exon1 (hsHTTexon1-Q72) or full-length (hsHTT-Q73) of human mutant HTT mRNA were transfected 24 hours after siRNA transfection, and their levels were measured after another 48 hours (12 technical replicates from 3 biological replicates). For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by two tailed unpaired t tests, ****P < 0.0001; n.s.: P > 0.05.
Figure 5. HuR interacts with #105708 site in exon 11 of the HTT mRNA.

(A) RT-qPCR quantifications of HuR-bound exogenously expressed HTT mRNA 5’ fragment (exon 1–10 or exon 1–11) levels in the wild-type mouse striatal cells (STHdhQ7/Q7) by RNA-IP (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. HuR interacted with exon 1–11 but not exon 1–10, suggesting that exon 11 contains the HuR-binding site. (B) Similar to (A), but in HD cells (STHdhQ7/Q111) transfected with cDNA plasmids expressing EGFP mRNAs containing different candidate binding sites in its 3’ UTR region (12 technical replicates from 3 biological replicates). The EGFP mRNA with #105708 site sequences (mouse or human) showed interaction with HuR, suggesting that #105708 (located in exon 11 of HTT mRNA) is a potential HuR-binding site. (C) A representative R-EMSA gel image of different Cy3-labelled RNA oligos of potential binding site sequences after co-incubation with purified HuR-MBP.His proteins (see Fig. S3). The first lanes in each group were loaded by the indicated RNA oligos alone. In the 2nd and 3rd lanes in each group, the indicated RNA oligos were incubated and loaded with purified HuR-MBP.His protein at different concentration ratios as indicated. Five repeats were performed, showing consistent results. HuR-binding with the RNA oligo leads to an apparent molecular weight shift to the top (dark arrows), due to mobility block caused by protein-binding. (D) Quantification of the ratio between firefly luciferase and renilla luciferase signals in HD mouse striatal cells (STHdhQ7/Q111) transfected with pmirGLO reporter plasmids (12 technical replicates from 3 biological replicates). The firefly/renilla signal ratio is an indicator of the level of firefly luciferase mRNA, of which the 3’ UTR region contained different sequences of potential HuR-binding sites. The HuR knockdown by siRNA caused lowering of the firefly/renilla signal ratio only for #105708-containing plasmids, suggesting that #105708 is likely a functional binding site. (E) RT-qPCR quantifications of HuR-bound endogenous mouse HTT (msHTT) mRNA levels in HD mouse striatal cells (STHdhQ7/Q111) transfected with pmirGLO plasmids expressing firefly luciferase mRNAs containing different potential binding sites (12 technical replicates from 3 biological replicates). The mRNAs with functional binding sites may compete with endogenous msHTT mRNA for HuR binding (illustrated in the schematic picture in the left panel). Thus, the RNA-IP signals of the corresponding samples could be reduced (right panel). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by two-tailed unpaired t tests, ****P < 0.0001; n.s.: P>0.05.
plasmid (pmirGLO). The 3’UTR of its expressed firefly luciferase mRNA contains the sequence of every single candidate binding site, whereas the renilla luciferase was expressed by an independent promoter in the same plasmid as an internal control. Thus, the firefly luciferase signal divided by the renilla luciferase signal (firefly/renilla signal) reflects the relative level of firefly luciferase mRNA containing each individual candidate binding site, and this ratio serves as readout for mRNA stability. We thus transfected these plasmids into the mouse HD cells (STHdh197/Q111) and tested the effect of HuR knockdown. Among all the candidate sequences tested in this reporter assay, only the #105708 (hs#105708 and ms#105708) sequences caused significantly decreased firefly/renilla signal after HuR knockdown (Fig. 5D), suggesting that the HuR-#105708 interaction is functional in regulating mRNA levels in the HD cells.

Finally, we tested if the #105708-containing mRNA may compete with endogenous HTT mRNA for HuR’s binding (Fig. 5E, see the schematic picture in the left panel). If so, #105708 RNA oligos may serve as potential reagents to decrease mutant HTT RNA stability for HD research and possible therapeutic intervention. Exogenous expression of #105708 containing firefly luciferase mRNA largely blocked the interaction between endogenous HuR proteins and the HTT mRNA, based on the RNA-IP experimental results (Fig. 5E, right panel), validating the competition effects of #105708. As controls, this phenomenon was not observed for firefly luciferase mRNAs containing other candidate HuR binding sequences (Fig. 5E, right panel). Collectively, HuR interacts with the HTT mRNA directly at the #105708 site in its exon 11 region in vitro and in the HD cells, and this interaction is functional in regulating target mRNA levels.

**HuR interacts with HTT mRNA in a mHTT-dependent manner via MAPK11**

Interestingly, the HuR-HTT mRNA interaction was not detected in wild-type cells, where mHTT was not present (Fig. 6A, mock; Fig. 4B, STHdh197/Q7 & Q16; Fig. 4C, hsHTT-Q23). This is consistent with the observation that HuR regulates both mutant and wild-type HTT mRNA in a mutant HTT-dependent manner (Fig. 2A–E). We thus tested if the HuR-HTT mRNA interaction is also dependent on the presence of mHTT. We transfected wild-type mouse cells (STHdh197/Q7) with plasmids expressing mHTT (hsHTTQ7/Q7) or its exon 1 fragment (hsHTTexon1-Q72) or the empty control plasmid (mock), and then performed the RNA-IP experiments similar to previously presented in Fig. 4C, but testing HuR’s interaction with the endogenous mouse HTT mRNA (mHTT) at this time (Fig. 6A). Expression of mHTT or its exon 1 fragment restored the originally missing interaction between HuR and the wild-type HTT mRNA (Fig. 6A, hsHTTQ7/Q7 and hsHTTexon1-Q72 compared with mock), confirming the mHTT-dependence of HuR’s binding to the HTT mRNA.

We further confirmed this by testing the functional consequence of the HuR-#105708 interaction using the dual-reporter assay. The firefly/renilla signal was not influenced by HuR knockdown in wild-type cells (STHdh197/Q7), even for #105708 containing pmirGLO plasmids (Fig. 6B), suggesting that the HuR-#105708 interaction is absent in the wild-type cells, different from the one observed in HD cells (Fig. 5D). However, when these cells were transfected with the mHTT exon 1 fragment (hsHTTexon1-Q72), the firefly/renilla signal in the #105708-containing group became sensitive to the HuR knockdown (Fig. 6C), confirming that mHTT expression is sufficient to drive the HuR-#105708 interaction. Taken together, the interaction between HuR protein and HTT mRNA is dependent on the presence of mHTT.

HuR locates in both the nuclei and cytoplasm, and shuttles between the two cellular compartments [17]. Since HuR mainly locates in the nuclei but stabilize mRNA in the cytoplasm [18], we hypothesize that it distributes more in the cytoplasm in the HD cells or the presence of mHTT, so that it interacts and regulates the HTT mRNA in an mHTT-dependent manner. Consistent with this hypothesis, the cytoplasmic localization of HuR is significantly higher in the mouse HD cells (STHdh197/Q111) compared to the one in wild-type cells (STHdh197/Q7), based on the Western-blots of cytoplasmic versus nuclear fractions of these cells (Fig. 7A). A similar phenomenon was observed in vivo in the striatal tissue from 6.5-month-old HD versus wild-type mice (Fig. 7B). To confirm the enhanced cytoplasmic localization of HuR, we immunostained the HuR protein in wild-type (STHdh197/Q111) versus HD (STHdh197/Q111) cells to investigate its cellular distribution by high-content confocal imaging technology. Imaging analysis of thousands of cells showed that the cytoplasmic to nuclear ratio of HuR protein is significantly higher in the HD than the wild-type cells (Fig. 7C), consistent with the biochemical fractionation experiments (Fig. 7A, B). Finally, to functional characterize the increased cytoplasmic localization of HuR in the HD cells, we performed RNA-IP in both wild-type and HD cells to test the HuR-bound HTT mRNA levels in nuclear versus cytoplasmic fractions. The cytoplasmic HuR-bound HTT mRNA level was significantly higher in the HD cells than the wild-type ones (Fig. 7D), consistent with the increased cytoplasmic localization of HuR in HD cells.

We then investigated the potential mechanism of the higher cytoplasmic localization of HuR in the HD cells. Our previous work identified MAPK11 as a positive modulator of HTT mRNA stability [19] and it is also activated by mHTT [20]. In addition, MAPK11 belongs to the p38 MAPK family, which are able to enhance HuR levels and activate cytoplasmic translocation of HuR [21], although the exact member(s) of this family that makes a major contribution to this function is yet to be identified. We thus hypothesized that mHTT may activate MAPK11 to enhance the cytoplasmic localization of HuR, which may then interact with and stabilize the HTT mRNA.

To test this hypothesis, we performed epistatic experiments to test if HuR is downstream of MAPK11 in regulating HTT mRNA levels. Consistent with our previous observation [19], knockdown of mouse MAPK11 (msMAPK11) significantly reduced HTT mRNA levels in the HD cells (STHdh197/Q111) (Fig. 8A). Meanwhile, knockdown of HuR largely abolished this effect, suggesting that HuR is downstream of MAPK11 in regulating HTT mRNA levels (Fig. 8A). Consistently, lowering HuR by knockdown or heterozygous knockout significantly...
A  RNA-IP in WT cells (STHdh\textsuperscript{Q7/Q7})

B  pmirGLO transfected WT cells (STHdh\textsuperscript{Q7/Q7})

C  pmirGLO + HTT exon1-Q72 transfected WT cells (STHdh\textsuperscript{Q7/Q7})
ameliorated MAPK11’s effects on the HTT mRNA stability in both mouse (STHdh<sup>Q7/Q111</sup>) and human HD cells (Q47) (Fig. 8B–D), confirming that HuR is likely downstream of MAPK11 in stabilizing the HTT mRNA.

We thus further investigated whether MAPK11 influences HuR’s cytoplasmic localization. MAPK11 knockdown significantly reduced cytoplasmic localization of HuR based on the Western-blots of cytoplasmic versus nuclear fractions of mouse HD cells STHdh<sup>Q7/Q111</sup> (Fig. 9A), illustrating that MAPK11 regulates the nuclei-cytoplasm distribution of HuR. Consistently, knockout of the mouse MAPK11 (msMAPK11<sup><small>−/−</small></sup>) significantly reduced the cytoplasmic/nuclear ratio of HuR in the 6.5-month-old HD mouse (Hdh<sup>Q140/Q140</sup>) striatal tissue as well, confirming that MAPK11 regulates HuR’s cytoplasmic localization as well (Fig. 9B). Consistent with this, MAPK11 knockdown reduced HuR cytoplasmic localization (Fig. 9C) as well as the cytoplasmic HuR-bound HTT mRNA level (Fig. 9D, in which the IPed msHTT mRNA in the cytoplasmic fraction of HD cells was obviously lowered by Mapk11<sub>−/−</sub> transfection comparing to Neg<sub>−/−</sub> transfected controls) in the HD cells. Taken together, the most likely interpretation of our data is that the presence of mHTT activates MAPK11 [19,20], which then induces cytoplasmic translocation of HuR. HuR then binds to the #105708 sequence in exon 11 of HTT mRNA and stabilizes it (Fig. 10). Thus, MAPK11 or HuR knockdown destabilizes the HTT mRNA in an mHTT-dependent manner.

Discussion

In this work, we identified HuR as a novel RBP that modulates HTT mRNA stability in HD cells. To our knowledge, it is the first reported RBP that interacting with and stabilizing the HTT mRNA.

The interaction between HuR and HTT mRNA is non-canonical in several ways, which may provide additional insights into HuR biology. It interacts with a non-AU-rich sequence in the coding region, instead of the canonical ARE in the 3’-UTR (Fig. 5). This type of interaction could be loose, compared to canonical ones, so that it does not interfere with protein translation. Meanwhile, this coding-region and non-AU-rich type of HuR interaction is still functional in stabilizing the target mRNA, consistent with a very recent HuR study [22].

Another interesting feature of HuR’s interaction with the HTT mRNA is that the interaction is dependent on the presence of mHTT DNA, RNA or protein (Fig. 6). The mHTT protein is likely the major contributor to activate HuR’s interaction with the HTT mRNA, because we utilized mixed CAGCAA sequences in the mHTT-expressing CDNA plasmids (Figs. 4C, D & 6B, C), which lacked the expanded CAG repeat sequences required to induce DNA replication errors or RNA foci formation or RNA phase separation [23,24]. Thus, it is more likely that the mHTT protein enabled HuR’s capability in regulating the HTT mRNA, possibly via activating MAPK11 (Figs. 8 & 9). Taken together, the mHTT-MAPK11-HuR-HTT mRNA pathway may form a positive feedback loop (Fig. 10), which may accelerate mHTT protein accumulation and contribute to HD progression over time.

The therapeutic potential of the pathway remains to be tested. While targeting the HTT mRNA by siRNA, shRNA or ASOs provides extremely promising strategies for HD treatment [25], these approaches require delivery of large biomolecules or viral particles such as AAV, which is challenging and expensive. In addition, these exogenously delivered large biomolecules or viruses might activate certain biological responses such as the interferon response that might be risky in certain circumstances. A very recent study has also shown that the CAG repeat number rather than polyQ length might determine the age of onset in HD [26], implying that the CAG repeat containing HTT mRNA may play a more important role than previously thought. Targeting the HTT mRNA by MAPK11/HuR provides a possible alternative approach for HD drug development. Both MAPK11 and HuR have reported inhibitors [27,28], which could be used to test the therapeutic potential of this pathway. In fact, our previous reports suggested that the MAPK11 inhibitor SB202190 may reduce mHTT levels to rescue HD-relevant phenotypes in the HD neurons [19], although the HuR inhibitors are unavailable to us. Meanwhile, further optimization of MAPK11 and/or HuR inhibitors may provide small compound drugs lowering the HTT mRNA to treat HD.

Besides the involvement of mHTT in HD, the wild-type HTT protein is also an important protein that is essential during development and plays critical roles in various cellular functions [29]. As an HTT mRNA modulator, HuR may participate in key biological functions via regulating the wild-type HTT as well. In our study, HuR-mediated modulation of HTT mRNA levels is dependent on the presence of mHTT, but it is possible that this regulation is activated under some other pathophysiological or even physiological conditions. In fact, MAPK11 and other p38 MAPKs are activated by various types of cellular stress such as oxidative, genotoxic, and osmotic stress and by proinflammatory cytokines occurring in many human diseases including neurodegenerative disorders other than HD [30]. Thus, HuR might stabilize the wild-type HTT mRNA in those disease neurons to protect them, considering the neuroprotective function of wild-type HTT [31]. Another interesting possibility is the HuR may play a role during development by modulating wild-type HTT. The p38 MAPK pathway is known to be activated during embryonic development [32], whereas wild-type HTT is also

Figure 6. HuR interacts with HTT mRNA in an mHTT-dependent manner. (A) RT-qPCR quantifications of the levels of endogenous mouse HTT (mHTT) mRNA bound with HuR in WT mouse striatal cells (STHdh<sup>Q7/Q0</sup>) transfected with empty vector (mock) or CDNA plasmids expressing full-length (hsHTT-Q7) or exon 1 (hsHTT exon1-G72) by RNA-IP (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 185 label was quantified as a baseline control to normalize the signals. (B) Similar to Fig. 5D, but in wild-type cells (STHdh<sup>Q7/Q0</sup>) (7 biological replicates). (C) Similar to Fig. 5D, but in wild-type cells (STHdh<sup>Q7/Q0</sup>) transfected with hsHTT exon1-G72 plasmid 24 hours before transfection of the pmirGLO reporter plasmids with the indicated potential binding sites (6 biological replicates). o-tailed unpaired t tests, ****P < 0.0001; n.s.: P > 0.05. For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by two-tailed unpaired t tests. **P < 0.01; ****P < 0.0001; n.s.: P > 0.05.
Figure 7. The nuclear/cytoplasmic distribution of HuR protein in wild-type versus HD cells or tissues.

(A) Representative Western-blots (from 4 biological replicates) of HuR protein levels in the nuclear or cytoplasmic fractions of wild-type (STHdh\(^{Q7/Q7}\)) or HD (STHdh\(^{Q7/Q111}\)) mouse striatal cells. β-Tubulin was blotted as a cytoplasmic loading control, and Lamin A/C as a nuclear loading control. These two control proteins were only detected in their localized fractions, confirming reliable nuclei/cytoplasm separation. The cytoplasmic/nuclear ratios of HuR signals were quantified and plotted (8 technical replicates from 4 biological replicates). (B) Similar to (A), but in the cytoplasmic and nuclear fractions of striatal tissue lysates from 6.5-month-old wild-type (Hdh\(^{Q7/Q7}\)) or HD (Hdh\(^{Q140/Q140}\)) mice (8 technical replicates from 4 biological replicates). (C) Representative images and quantifications of immunofluorescent staining of the distribution of HuR protein in wild-type (STHdh\(^{Q7/Q7}\)) or HD (STHdh\(^{Q7/Q111}\)) cells. The cytoplasmic/nuclear ratios of HuR signals were quantified for each cell and presented by beanplot (4 biological replicates with more than 10,000 cells for each replicate). (D) RT-qPCR quantifications of HuR-bound HTT mRNA levels in nuclear and cytoplasmic fractions of HD (STHdh\(^{Q7/Q111}\)) or wild-type (STHdh\(^{Q7/Q7}\)) cells detected by RNA-IP (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. For all plotted data, error bars represent mean and SEM. For all bean plots, the long black solid line represents the mean of each replicate, the grey dashed line represents the mean of all data points, and short black solid lines represent the outliers of original data. The statistical analysis was performed by two-tailed unpaired t tests, ***P < 0.001, ****P < 0.0001; n.s., P > 0.05.
**Figure 8.** MAPK11 is an upstream modulator of HuR and controls HuR’s regulation of HTT mRNA levels in HD cells.

(A) RT-qPCR quantifications of endogenous *msHTT* mRNA levels in HD mouse striatal cells (STHdh^{Q7/Q111}) transfected with the indicated siRNAs (12 technical replicates from 3 biological replicates). *msMAPK11* siRNA (grey bars) or the non-targeting control siRNA (Neg_si, white bars) were co-transfected with HuR siRNA (HuR_si) or the non-targeting control siRNA (Neg_si). *msHTT* mRNA levels were measured 48 hours after transfection.

(B) HTT mRNA degradation measurements in HD mouse striatal cells (STHdh^{Q7/Q111}) transfected with the indicated siRNAs for 36 hours, and then treated with actinomycin D (12 technical replicates from 3 biological replicates). The quantification of HTT mRNA degradation was then performed the same as in Fig. 3A, B. Data values (mean ± S.E.M.) have been indicated for each point.

(C) Similar to (B), but in immortalized human HD fibroblast (Q47) (12 technical replicates from 3 biological replicates). Data values (mean ± S.E.M.) have been indicated for each point.

(D) Similar to (B), but in pooled colonies of mouse HD cells (STHdh^{Q7/Q111}) with the heterozygous knockout of HuR (12 technical replicates from 3 biological replicates). Data values (mean ± S.E.M.) have been indicated for each point. For all plotted data, error bars represent mean and SEM. The statistical analysis was performed by two-way ANOVA (B-D) or two-tailed unpaired t tests (A). ****P < 0.0001; n.s., P > 0.05.
Figure 9. MAPK11 regulated nuclei/cytoplasm distribution of HuR protein in HD cells or tissues.
(A) Representative Western-blot and quantifications of cytoplasmic versus nuclear HuR protein levels in the HD mouse striatal cells (STHdh<sup>Q7/Q111</sup>) transfected with the indicated siRNAs (8 technical replicates from 4 biological replicates). (B) Representative Western-blot and quantifications of cytoplasmic versus nuclear HuR protein levels in the lysates of striatal tissues from 6.5-month-old HD (Hdh<sup>Q140/Q140</sup>) mice with or without msMAPK11 knockout (8 technical replicates from 4 biological replicates). (C) Similar as Fig. 7C, but in HD (STHdh<sup>Q7/Q111</sup>) cells transfected with the indicated siRNAs (4 biological replicates). (D) Similar as in Fig. 7D, but using HD cells transfected with the indicated siRNAs (12 technical replicates from 3 biological replicates). IgG was used as a negative control for the IP, and the 18S level was quantified as a baseline control to normalize the signals. For all plotted data, error bars represent mean and SEM. For all bean plots, the long black solid line represents the mean of each replicate, the grey dashed line represents the mean of all data points, the grey area represents the distribution of data, and short black solid lines represent the outliers of original data. The statistical analysis was performed by two-tailed unpaired t tests, ****P < 0.0001; n.s., P > 0.05.
an essential protein at this stage [33,34]. Thus, HuR might play an important role at this stage through regulating wild-type HTT levels.

Material and methods

Utilization of gene symbols

The official symbol of HuR is ‘ELAVL1’, but since most of publications in the literature used ‘HuR’, we also used HuR in this paper to be consistent with others. To clarify the species, we added ‘hs’ or ‘ms’ before the gene symbol to indicate human or mouse species, respectively. In a few places where the symbol stands for both human and mouse version, ‘hs’ or ‘ms’ was not added. We used the italic font for DNAs or RNAs, and the regular font for proteins.

Plasmid constructs

The human HTT-exon1 (hsHTTexon1-Q72) and full-length (hsHTT-Q23 & hsHTT-Q73) constructs were synthesized (Thermo Fisher Scientific) and subcloned into the pcDNA/Puro-CAG plasmid vector (The schematic models of inserted constructs are shown in Fig. S4A). The human HTT exon 1–10 and exon 1–11 constructs were cloned by PCR amplification from the hsHTT-Q73 and subcloned back to pcDNA/Puro-CAG plasmid vector. All these constructs have mixed CAGCAA sequences to express the polyQ sequence. The pFRT-to-eGFP-ELAVL1 (human HuR) (Addgene, #106105) construct was obtained from Addgene. The pmirGLO Dual-Luciferase miRNA target expression vector (Promega #E1330), and all of the dual-luciferase plasmids with specific HTT target site (#105708, #105717, etc.) were constructed by ligating the annealed oligos to the original backbone at the 3’UTR region of the firefly luciferase (Fig. S4B). The HuR-MBP.His plasmid was cloned by PCR amplification of the HuR cDNA from pFRT-to-eGFP-ELAVL1 plasmid and then ligated it to the pMal-c2x (Addgene, #75286; we have inserted C-terminal poly-His and the TEV protease cleavage site expressing sequence to the 3’) plasmid.

Cell culture

The patient-derived Q47 fibroblasts were described previously [19]. Human fibroblasts cell lines (GM03868 – Q45; GM21757 – Q68) and STHdh (CH00097 – Q7/Q7 and CH00096 – Q111/Q7) were obtained from Coriell Cell Repositories. HEK293T and STHdh cells were cultured in DMEM (Thermo Fisher Scientific, #11965) with 10% FBS (Thermo Fisher Scientific, #10082), whereas human fibroblasts were cultured in DMEM with 15% FBS. All the STHdh cells were maintained at 33°C incubator with 5% CO₂, and HEK293T and human fibroblasts were kept at 37°C with 5% CO₂. The cells were tested for mycoplasma contamination every month.

The homogeneous time-resolved fluorescence (HTRF) assay

The HTRF assays were performed similarly to those previously described [35]. Basically, the cells were lysed in the lysis buffer (1x PBS with 0.4% Triton X-100 and 1x protease inhibitor final) and the HTT protein was detected with 2B7/MW1 antibody pair. For all samples, the total protein concentration (by BCA, Beyotime, #P0009) was measured to correct the loadings. Different protein concentrations or cell numbers per well were tested to ensure that the signals were

Figure 10. A schematic model illustrating mHTT-dependent MAPK11-HuR mediated regulation of HTT mRNA stability. The presence of mHTT activates MAPK11 (ref. [19,20]), leading to translocation of HuR protein to the cytoplasm (ref. [21]) (Figs. 7 & 9) and its interaction with HTT mRNA in its exon 11 at the #105708 site (Figs. 4 & 5) to stabilize it in a mHTT-dependent manner (Figs. 2, 6 & 8B–D).
in the linear range. Background corrections were performed by subtracting the background signals from blank samples.

**cDNA and siRNA transfection**

All cDNAs were transfected with lipofectamine 3000 (Life Technologies, #L3000), whereas siRNAs were transfected into the STHdh cells with lipofectamine 2000 (Life Technologies, #11668). Transfection steps were performed according to the manufacturer’s protocol. Cells were collected at different timelines for diverse experimental purpose. Briefly, cells were collected 24 hours after plasmid transfection for dual-luciferase detection, or 48 hours after cDNA or siRNA transfection for qPCR or RNA-IP, or 72 hours after siRNA transfection for Western blot or HTRF. In some experiments (Figs. 2E, 4D & 5D), the cells were first transfected with siRNA and then with cDNA. In this case, the siRNA was first transfected for 24 hours. The culture medium was then changed, and the cDNA transfection was performed. The cells were then collected for experiments 48 hours after cDNA transfection. All siRNAs have been validated by qPCR and/or western blots for target knockdown. siRNA target sequences are shown as below: HuR_siRNA 1 (both human and mouse): AAGAGGCAATTACCAGTTTCA; HuR_siRNA 2 (both human and mouse): GGATGAAGTGGCAGACA; MAPK 11_siRNA (human): CACCGATGTATGCACTGCACA; Mapk 11_siRNA (mouse): CGCCAGAGATCATGC TAA. Neg_siRNA was purchased commercially (QIAGEN, #1027280).

**RT-qPCR and mRNA stability measurements**

mRNA levels were determined by RT-qPCR, and the protocol was followed as previously described. Briefly, RNA from siRNA-transfected and/or actinomycin D-treated cells was extracted using RNaseq Prep Pure Cell/Bacteria Kit according to the manufacturer’s instruction (Tiangen, #DP430). cDNA was obtained by reverse transcription with random primers using the FirstQuant RT Kit (Tiangen, #KR106). qPCR was then performed using SYBR Green Realtime PCR Master Mix (Toyobo, #QPK-201) with no reverse-transcriptase controls used to ensure the specificity of the signals. All the primers were tested with a standard curve (Fig. S5).

For mRNA stability measurements, the cultured cells were treated with actinomycin D (5 μg/mL, Sigma, #A9315) that inhibits transcription at time 0, and then collected at indicated time points for quantification of different mRNAs by RT-qPCR. The qPCR primer sequences are (note that STHdh Q7/Q111 cells express the mouse wild-type HTT mRNA, as well as a mouse-human chimera HTT mRNA with its exon1 and part of intron 1 replaced by the human version with expanded CAG repeats (111 CAG), but the msHTT qPCR primers targeting the 3’ sequences can detect both): hs18S-forward: GGTTGAGGCTTCCAGTGCAA; hsHuR-forward (human): CCCTCTGAGCAAAACCTGTAGT; msHuR-forward (mouse): CACCCACGGCCACAGGATT; msHuR-reverse (mouse): CGGGGACATTGCACACCAGAA; EGFP-forward: TATATCATGCGCCGCAAGCG; EGFP-reverse: GTTTGGGATGATTTTAGGTGTT; exon 1–11 of mutant HTT-forward: CACCGCTGAAGAGAGAG; exon 1–11 of mutant HTT-reverse: AGGACCCCCTTCCAGCTA; hsHTT exon 1-Q72-forward: CCTAGGCAACAATCTCTC; hsHTT exon 1-Q72-reverse: CGTATGGGTATCCTGTCGAG

**RNA immunoprecipitation (RNA-IP)**

To perform RNA-IP, protein G magnetic beads (Bio-rad, #161-4023) were first blocked with 5% BSA and 2 μg/mL tRNA on a roller for 1.5 hours at 4 °C. Then mixed the beads with antibody in RIP buffer (100 mM KCl + 25 mM Tris-HCl (pH = 7.4) + 1 M EDTA + 0.5 mM DTT + 0.5% NP-40 + 1× RNase inhibitor (Thermo Fisher Scientific, #N8080119) + 1× Complete protease inhibitor (Roche, #04693132001)) overnight on a roller at 4 °C. The next day, cell samples were collected and lysed by the RIP buffer, and one-third of the sample was kept as the input control before pull-down by the antibody-conjugated beads. The lysates were incubated with the beads overnight on a roller at 4 °C, and then the beads were washed by cold RIP Buffer followed by 1× PBS once. The RNA pulled down by the beads was then isolated by RNA simple Total RNA kit (TIANGEN, #DP419). The mRNA level was then measured and analysed by RT-qPCR, as illustrated above.

**RNA electrophoretic mobility shift assay (R-EMSA)**

The human HuR protein was purified for the R-EMSA experiments. To obtain the recombinant purified HuR protein, HEK293T cells were transfected with the HuR-MBP.His plasmid, and then collected after 48h. The cellular protein was then extracted by NiA Buffer (20 mM imidazole + 5% glycerol + 150 mM NaCl + 100 mM Tris-HCl (pH = 7.4) + 1 × Complete protease inhibitor (Roche, #04693132001)), and then loaded onto the Ni-NTA superflow column (QIAGEN, #30622). Column bound proteins were then eluted by NiB Buffer (300 mM imidazole + 5% glycerol + 150 mM NaCl + 100 mM Tris-HCl (pH = 7.4) + 1 × Complete protease inhibitor (Roche, #04693132001)). Finally, the purified proteins were concentrated to approximately 2 mg/mL in 50 mM HEPES buffer with 100 mM NaCl for further analysis, and the purity of HuR protein was confirmed by coomassie brilliant blue staining. To perform RNA-EMSA, the Cy3-labelled RNA oligos (4μM) were first heated to 95 °C and cooled down to 25 °C at room temperature. Then the titrated HuR protein (2 mg/mL) and RNA oligos were dissolved in the EMSA interaction buffer (1 × R-EMSA Binding Buffer (Thermo Fisher Scientific, #20158) + 5% glycerol + 100 μg/mL tRNA+1× RNase inhibitor (Thermo Fisher Scientific, #N8080119)), and then incubated for 30 min at room temperature (25 °C). The reaction mixture was then loaded onto a 6% acrylamide native gel and run the electrophoresis at 4 °C. Shift band intensities were captured by Typhoon FLA-9000 (GE).
Nuclear/cytoplasmic protein extraction and Western-blots

The nuclear/cytoplasmic protein extraction was performed in the same way as previous reports [36]. Cell pellets were collected and lysed on ice for 5 min in lysis buffer (50 mM Tris-HCl (pH = 7.4) + 10 mM NaCl + 0.5% NP-40 + 0.25% Triton X-100 + 1 mM EDTA + 1× Complete protease inhibitor (Roche, #04693132001)) and centrifuged at 3000 × g at 4 °C for 5 min. The nuclear compartments were in the precipitates, and the supernatants then contained cytoplasmic proteins. Then the precipitates were washed with the lysis buffer once and resuspended in the NET Buffer (20 mM HEPES + 1.5 mM MgCl₂ + 0.5 M NaCl + 0.2 mM EDTA + 20% glycerol + 1% Triton X-100), sonicated for 0.8 minutes at 17 W, and then centrifuged at 10000 × g at 4 °C for 10 min. The supernatants collected at this time then contain specific proteins from the nuclei. Western-blots for the nuclei marker Lamin A/C and cytoplasmic marker β-Tubulin were performed to confirm the nuclei/cytoplasm separation (Figs. 7A, B, & 9A, B). For whole cell lysates, 10⁶ cells were collected and resuspended in 100 μl PBS-Triton X Buffer (1 × PBS + 1% Triton X-100) with 1× Complete protease inhibitor (Roche, #04693132001) on ice for 30 min. The suspension was sonicated for 0.8 minutes at 17 W and then centrifuged at 20000 × g at 4 °C for 10 min. The supernatant was kept as the total cell protein. All the protein samples were then loaded and transferred onto nitrocellulose membranes for Western-blots. Commercially purchased antibodies include anti-HuR (Proteintech, #11910-1-AP), anti-β-Tubulin (Abcam, #ab60464), anti-Lamin A/C (Proteintech, #10298-1-AP), anti-p38-β2 (Invitrogen, #33-8700). The specificity of all antibodies has been validated by previous reports or our knockdown or knockout experiments.

Immunofluorescence and high-content imaging

For immunofluorescence, cultured cells were washed three times with 1× PBS and then fixed in 4% paraformaldehyde (PFA) for 15 minutes, followed by permeabilizing in 0.5% TritonX-100 for 10 min. The cells were then blocked in 4% BSA + 0.1% Triton X-100 in 1× PBS and incubated with primary antibodies at 4°C overnight, and then washed with blocking buffer and incubated with secondary antibody at room temperature for 1 h. The samples were then washed, 0.5 mg/ml DAPI (Beyotime Biotechnology, #C1002) was added for 5 min before the cells were final washed and stored in 1× PBS. The images of samples were scanned and then analysed by the high-content imaging system Operetta CLS (PerkinElmer) and its accompanying software Harmony 4.8. The bean plots of the analysed data were generated by online application: http://shiny.chemgrid.org/boxplot, which was developed according to the description of Nature Method [37].

Statistical analysis

Statistical comparisons between two groups were conducted by the two-tailed unpaired Student’s t-tests. Statistical comparisons among multiple groups were conducted by ANOVA tests. Significance was established at P < 0.05. In all graphs, error bars indicate SEM, and the biological replicate numbers are indicated on top of each bar and/or as the n numbers in the legends. The statistical powers for all analysis were calculated and confirmed to be > 80%. Data were excluded when there were clear indications of the artefact or experimental failures, such as contamination, transfection/infection failure, etc.

Acknowledgments

We would like to thank Dr. Yang Li for kindly providing the pmirGLO plasmids. We would like to thank Drs. Jianhua Gan and Binglian Zheng for providing insightful discussion and technical supports.

Data availability

The CLIP-seq data analysis results were obtained from the previously published database StarBase, which is available at: http://starbase.sysu.edu.cn/.

The RBP expression was analyzed by the BioGPS database, which is available at: http://biogps.org/#goto=welcome.

The accession numbers of the genes relevant to this study include: Htt-huntingtin [Homo sapiens] (hsHTT); Gene ID 3064. MAPK11 [Mus musculus] (msMAPK11); Gene ID 15194. Lamin A/C [Homo sapiens] (hsLaminAC); Gene ID 1994. SOD1 [Mus musculus] (msSOD1); Gene ID 5600. Htt-huntingtin [Homo sapiens] (hsHTT); Gene ID 19094.

All the gene information is available at: https://www.ncbi.nlm.nih.gov/gene.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Project Supported by National Natural Science Foundation of China [31970747, 31601105, 81925012], Science and Technology Commission of Shanghai Municipality [18410722100], Shanghai Municipal Science and Technology Major Project [No.2018SHZDZX01] and ZLab, and Hereditary Disease Foundation for funding supports. Funding for open access charge: National Natural Science Foundation of China.

ORCID

Jian Wang http://orcid.org/0000-0002-2748-395X
Boxun Lu http://orcid.org/0000-0002-1675-9340

References

[1] The Huntington’s Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell. 1993;72:971–983.
[2] Rubinsztein DC, Carmichael J. Huntington’s disease: molecular basis of neurodegeneration. Expert Rev Mol Med. 2003;5:1–21.
[3] Feng X, Luo S, Lu B. Conformation polymorphism of polyglutamine proteins. Trends Biochem Sci. 2018;43:424–435.
[4] Rodriguez-Lebron E, Denovan-Wright EM, Nash K, et al. Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington’s disease transgenic mice. Mol Ther. 2005;12:618–633.
[5] Harper SQ, Staber PD, He X, et al. RNA interference improves motor and neuropathological abnormalities in a Huntington’s disease mouse model. Proc Natl Acad Sci U S A. 2005;102:5820–5825.

[6] DiFiglia M, Sena-Esteves M, Chase K, et al. Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc Natl Acad Sci U S A. 2007;104:17204–17209.

[7] Kordasiewicz HB, Stanek LM, Wancewicz EV, et al. Sustained therapeutic reversal of Huntington’s disease by transient repression of huntingtin synthesis. Neuron. 2012;74:1031–1044.

[8] Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, et al. Targeting huntingtin expression in patients with Huntington’s disease. N Engl J Med. 2019;380:2307–2316.

[9] Chung DW, Rudnicki DD, Yu L, et al. A natural antisense transcript at the Huntington’s disease repeat locus regulates HTT expression. Hum Mol Genet. 2011;20:3467–3477.

[10] Ito D, Hatano M, Suzuki N. RNA binding proteins and the pathological cascade in ALS/FTD neurodegeneration. Sci Transl Med. 2017;9:eaah5436.

[11] Yu S, Liang Y, Palacino J, et al. Drugging unconventional targets: insights from Huntington’s disease. Trends Pharmacol Sci. 2014;35:53–62.

[12] Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-cRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014;42:D92–97.

[13] Wu C, Jin X, Tsuang G, et al. BioGPS: building your own mash-up of gene annotations and expression profiles. Nucleic Acids Res. 2016;44:D313–316.

[14] Trettel F, Rigamonti D, Hilditch-Maguire P, et al. Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Hum Mol Genet. 2000;9:2799–2809.

[15] Peng SS, Chen CY, Xu N, et al. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. Embo J. 1998;17:3461–3470.

[16] Ke Y, Han Y, Guo X, et al. PARP1 promotes gene expression at the post-transcriptional level by modulating the RNA-binding protein HuR. Nat Commun. 2017;8:14632.

[17] Fan XC, Steitz JA. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases in the vivo stability of ARE-containing mRNAs. Embo J. 1998;17:3448–3460.

[18] Fan XC, Steitz JA. HNS, a nuclear-cytoplasmic shuttling sequence in HuR. Proc Natl Acad Sci U S A. 1998;95:15293–15298.

[19] Yu M, Fu Y, Liang Y, et al. Suppression of MAPK11 or HIPK3 reduces mutant Huntingtin levels in Huntington’s disease models. Cell Res. 2017;27:1441–1465.

[20] Taylor DM, Moser R, Regulier E, et al. MAP kinase phosphatase 1 (MKP-1/DUSP1) is neuroprotective in Huntington’s disease via additive effects of JNK and p38 inhibition. J Neurosci. 2013;33:2313–2325.

[21] Joassard OR, Belanger G, Karmouch J, et al. HuR mediates changes in the stability of AChR beta-subunit mRNAs after skeletal muscle denervation. J Neurosci. 2015;35:10949–10962.

[22] Xiao L, Li X, Chung HK, et al. RNA-binding protein HuR regulates paneth cell function by altering membrane localization of TLR2 via posttranscriptional control of CNPY3. Gastroenterology. 2019;157:731–743.

[23] Jain A, Vale RD. RNA phase transitions in repeat expansion disorders. Nature. 2017;546:243–247.

[24] Swami M, Hendricks AE, Gillis T, et al. Somatic expansion of the Huntington’s disease CAG repeat in the brain is associated with an earlier age of disease onset. Hum Mol Genet. 2009;18:3039–3047.

[25] Tabrizi SJ, Ghosh R, Leavitt BR. Huntington lowering strategies for disease modification in Huntington’s disease. Neuron. 2019;102:899.

[26] Genetic Modifiers of Huntington’s Disease Consortium. Electronic address, g.h.m.h.e. and Genetic Modifiers of Huntington’s Disease, C. CAG repeat not polyglutamine length determines timing of Huntington’s disease onset. Cell. 2019;178:887–900 e814.

[27] Frantz B, Klett T, Pang M, et al. The activation state of p38 mitogen-activated protein kinase determines the efficiency of ATP competition for pyridinylimidazole inhibitor binding. Biochemistry. 1998;37:13846–13853.

[28] Lang M, Berry D, Passecker K, et al. HuR small-molecule inhibitor elicits differential effects in adenomatosis polyposis and colorectal carcinogenesis. Cancer Res. 2017;77:2424–2438.

[29] Souda F, Humbert S. The biology of Huntington. Neuron. 2016;89:910–926.

[30] Kim EK, Choi EJ. Compromised MAPK signaling in human diseases: an update. Arch Toxicol. 2015;89:867–882.

[31] Buren C, Wang L, Smith-Dijak A, et al. Region-specific pro-survival signaling and global neuronal protection by wild-type Huntington. J Huntington Dis. 2014;3:365–376.

[32] Bradham C, McClay DR. p38 MAPK in development and cancer. Cell Cycle. 2006;5:824–828.

[33] Zeitlin S, Liu JP, Chapman DL, et al. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington’s disease gene homologue. Nat Genet. 1995;11:155–163.

[34] Nasir J, Floresco SB, O’Kusky JR, et al. Targeted disruption of the Huntington’s disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell. 1995;81:811–823.

[35] Lu B, Al-Ramahi I, Valencia A, et al. Identification of NUB1 as a suppressor of mutant Huntington toxicity via enhanced protein clearance. Nat Neurosci. 2013;16:562–570.

[36] Tollervey JR, Curk T, Rogelj B, et al. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci. 2011;14:452–458.

[37] Spitzer M, Wildenhaus J, Rapsilber J, Tyers M. Boxplotr: a web tool for generation of box plots. Nature Methods. 2014;11:121–122. DOI: 10.1038/nmeth.2811