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Original Article

Truncated N-terminal huntingtin fragment with expanded-polyglutamine (htt552-100Q) suppresses brain-derived neurotrophic factor transcription in astrocytes

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Although huntingtin (htt) can be cleaved at many sites by caspases, calpains, and aspartyl proteases, amino acid (aa) 552 was defined as a preferred site for cleavage in human Huntington disease (HD) brains *in vivo*. To date, the normal function of wild-type N-terminal htt fragment 1-552 aa (htt552) and its pathological roles of mutant htt552 are still unknown. Although mutant htt (mhtt) is also expressed in astrocytes, whether and how mhtt contributes to the neurodegeneration through astrocytes in HD remains largely unknown. In this study, a glia HD model, using an adenoviral vector to express wild-type htt552 (htt552-18Q) and its mutation (htt552-100Q) in rat primary cortical astrocytes, was generated to investigate the influence of htt552 on the transcription of brain-derived neurotrophic factor (BDNF). Results from enzyme linked immunosorbent assay showed that the level of BDNF in astrocyte-conditioned medium was decreased in the astrocytes expressing htt552-100Q. Quantitative real-time polymerase chain reaction demonstrated that htt552-100Q reduced the transcripts of the BDNF III and IV, hence, repressed the transcription of BDNF. Furthermore, immunofluorescence showed that aggregates formed by htt552-100Q entrapped transcription factors cAMP-response element-binding protein and stimulatory protein 1, which might account for the reduction of BDNF transcription. These findings suggest that mhtt552 reduces BDNF transcription in astrocytes, which might contribute to the neuronal dysfunction in HD.

Keywords Huntington's disease; astrocytes; brain-derived neurotrophic factor; huntingtin; transcription

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Introduction

Huntington disease (HD) is a dominantly inherited neurodegenerative disorder resulted from an expansion of polyglutamine (polyQ) tract in the N-terminus of huntingtin (htt) [1,2]. The implicated protein htt is a 348-kDa cytoplasmic protein widely expressed throughout the brain and peripheral organs [3]. Htt was the first neuronal protein shown to be a caspase substrate, with defined sites for caspase-2 at amino acid (aa) 552, for caspase-3 at aa513 and 552, and for caspase-6 at aa586 [4–6]. In addition, htt can also be cleaved by calpains and aspartyl proteases *in vitro* [7–9]. However, *in vivo*, aa552 was defined as a preferred site for caspase cleavage in human HD brains, YAC72 transgenic mice brains, and control human and murine brains [10].

While full-length wild-type htt has been shown to be neuroprotective in a variety of *in vitro* and *in vivo* models [11–13], the N-terminal cleavage fragments liberated in HD patients lead to cellular dysfunction and neuronal death [14–17]. Previously, the N-terminal cleavage fragments used in HD study were mostly artificial such as N-terminal htt fragment containing exons 1 and 2 or other fragments produced randomly by restriction enzymes. Since these fragments *per se* did not exist in HD patient's brain, the findings based on these artificial fragments might not mimic the real pathological conditions of HD in some aspects [18]. To date, the normal function of wild-type htt552 and pathological roles of its mutation are still little known. Therefore, much more attention should be paid on the function of htt552 fragment, which exists in brain naturally.

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor (NGF) family in mammalian

brain, which is widely expressed in the adult mammalian central nervous system, and is particularly abundant in the hippocampus and cerebral cortex [19]. Many studies showed that BDNF levels decreased in the brain of HD patients, especially in striatum [20]. The decrease of BDNF at protein levels has been attributed to its reduction at transcription levels. Several studies showed that wild-type full-length htt stimulated BDNF gene transcription by inhibiting the repressor element 1/neuron-restrictive silencer element (RE1/NRSE) within BDNF promoter II [13,21]. While mutant htt (mhtt) fragments in animal models of HD such as YAC mice and R6/2 mice reduced the levels of BDNF mRNA III and IV. The mechanisms underlying the reduction are related to the dysfunction of some transcription factors [13,22,23]. As BDNF promoter III has a cAMP-response element (CRE), the dysfunction in CRE activity may account for its reduced transcriptional activity in HD [24,25]. Existing evidence indicates that the key protein in this event is the cAMP-response element-binding protein (CBP), which is affected by mhtt [26]. Another study showed that the reduced transcription of BDNF promoter IV was related to the stimulatory protein 1 (Sp1) [27]. Mhtt sequestered Sp1, which thus might block its physiological interaction with TAFII-130 and cause reduced transcriptional activity [28,29]. However, there is no report on the effects of wild-type htt552 or its mutation on the transcription of BDNF.

In the central nervous system, astrocytes are the largest cell population and play multiple roles in regulating neuronal functions. Among their multiple functions, astrocytes can synthesize and release some important neurotrophic factors including NGF, BDNF, ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF). All of them are involved in neuronal survival and function [30–32]. Existing evidence reveals that mhtt decreases cytokine secretion in HD animal model [33]. Chou *et al*. found mhtt in R6/2 mice reduced the expression of chemokine [(C-C motif) ligand 5 (CCL5)/regulated on activation of normal T cell expressed and secreted at the transcriptional level as well as retained it inside astrocytes [34]. Mhtt may affect the production of other chemokines or neurotrophic factors especially BDNF in astrocytes, although this possibility remains to be explored.

In the present study, we investigated the influences of wild-type htt552 (htt552-18Q) and its mutation (htt552-100Q) on the transcription of BDNF in primary cultured astrocytes. We found that htt552-100Q reduced BDNF transcription, through the disruption of transcription factors CBP and Sp1 in astrocytes. Such dysregulation of BDNF transcription in astrocytes greatly reduced the trophic functions of astrocytes to neurons, which might contribute to the neuronal injury in HD.

Materials and Methods

Primary cortical astrocyte cultures

Primary astrocytes were purified from 1 to 2 days postnatal Sprague–Dawley rats according to previously described method [35]. In brief, cerebral cortex was isolated, cut into pieces, and collected in ice-cooled phosphate-buffered saline (PBS). The tissue was dissociated with 0.25% (*v*/*v*) trypsin (Sigma, St Louis, USA) digestion and trituration with Pasteur pipette in Dulbecco's modified Eagle media: nutrient mixture F-12 (DMEM/F12; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS; Hangzhou Sijiqing Biological Company, Hangzhou, China). After passing through a 40-μm pore nylon mesh, cells were centrifuged (1000 *g*) at 4°C for 5 min and resuspended in 10 ml DMEM/F12 containing 20% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. After plating in flasks for 30 min, cells in the supernatant were plated at a density of 1×10^5 cells/cm^2 onto flasks coated with 0.01% poly-L-lysine (Sigma). Cells were incubated in a humidified atmosphere with 5% CO_2 at 37°C, and medium was changed twice a week. When cells reached 90% confluence, microglia, and other kinds of cells were removed by standard shaking. The remaining cells were then trypsinized, plated in new flasks, and cultured until the cells reached confluence again. After three repetitions of this procedure, a pure preparation of astrocytes was obtained.

Adenoviral vector construction and infection

The N-terminal of wild-type (18Q) and mutant (100Q) htt of 3-kb cDNAs, with a stop codon after 552 aa, was cloned into pDC316 adenovirus shuttle plasmid. Wild-type htt and mhtt cDNAs were excised from their parental vectors using *Bam*HI/*Xba*I, and ligated to *Bam*HI/*Xba*I-digested pUC18, an intermediate vector. Then these cDNAs were ligated to *Bgl*II/*Sal*I-digested pDC316. Two independent adenovirus shuttle plasmids, pDC316-htt-18Q-552stop and pDC316-htt-100Q-552stop, were obtained.

Two independent adenoviruses, Ad-htt-18Q-552stop and Ad-htt-100Q-552stop, were obtained through co-transfecting T293A cells with the backbone plasmid pBHG10 and the shuttle plasmids: pDC316-htt-18Q-552stop or pDC316-htt-100Q-552stop. Cytopathic effects happened at 7th day. The cells were collected at 10th day to obtain the first generation adenovirus. Then the first generation adenoviruses were proliferated in T293 cells. The viruses are named Ad-htt-18Q-552aa and Ad-htt-100Q-552aa, respectively. The fourth generation adenovirus was used in the later experiments. The titers of viruses were 5×10^9 and 7×10^9, respectively. In addition, Ad-null-GFP adenoviral

vector was generated and proliferated using the same protocol.

After purification procedure, cortical astrocytes were seeded onto new plates to be used for virus infection. At 80% confluence, cells were incubated in DMEM/F12 medium with 5% FBS containing adenoviral vectors with indicated titers. The virus containing medium was removed 5 h later and replaced with DMEM/F12 supplemented with 10% FBS. Cell cultures infected with adenoviral vectors (18Q, 100Q, and Null) and control cells (without infection) were cultured for an additional 48 h and then total proteins or RNAs were harvested for western blot analysis or quantitative real-time polymerase chain reaction (qRT-PCR).

Preparation and collection of astrocyte-conditioned medium

To prepare astrocyte-conditioned medium (ACM), primary astrocytes were cultured at the same density (1.5×10^6 in 25-cm^2 flasks) in DMEM/F12 supplemented with 10% FBS for 2 days, then infected with adenoviral vectors with indicated titers as shown above. The virus containing medium was removed 5 h later and washed twice with PBS. Cell cultures infected with adenoviral vectors (18Q, 100Q, and Null) and control cells were cultured in serum-free DMEM/F12 for an additional 24, 48, 72, and 96 h. The ACM was then collected, centrifuged at 1000 *g* for 5 min to remove cell debris, and stored at −80°C for further analysis.

Enzyme linked immunosorbent assay

Protein levels of BDNF in ACM were determined using a chemikine BDNF enzyme linked immunosorbent assay (ELISA) kit (Millipore Corporation, Billerica, USA) following the manufacturer's protocol. Briefly, samples or standards (100 μl) were added to the microplates, incubated at 4°C overnight, and washed extensively, followed by a 3-h incubation with the biotinylated mouse anti-BDNF monoclonal antibody and a 1-h incubation with streptavidin-horseradish peroxidase (HRP) plus substrate for signal development. The optical density of each well was detected using a microplate reader (Microplate Reader; Benchmark, Pasadena, USA), and readings were subtracted from those at 540 nm. The amount of BDNF in each sample was calculated based on the standard curve prepared in the same experiment. Values were presented as the mean ± standard deviation of nine determinations from three different batches of ACM ($n = 3$).

Antibodies

Mouse anti-htt monoclonal antibody 2166 was purchased from Chemicon (Temecula, Riverside County, USA; dilution 1 : 2000); rabbit anti-htt antibody Ab1 was a generous gift from Dr Marian DiFiglia (Massachusetts General Hospital, Boston, USA; dilution 1 : 2000); CBP antibody was purchased from Santa Cruz (Santa Cruz, USA; dilution 1 : 1000); rabbit polyclonal anti-Sp1 was purchased from Millipore (Billerica, USA; dilution 1 : 2000); and β-actin antibody was purchased from Sigma (dilution 1 : 5000).

Western blot analysis

Astrocytes were rinsed with ice-cooled PBS and harvested. Five volumes of western blot lysing buffer (containing 20 mM Tris-HCl, pH 7.0, 20 mM EDTA, and 0.5% TritonX-100 supplemented with a cocktail of protease inhibitors containing 1 mM pefabloc, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) for each volume of cell pellets were added and incubated for 30 min on ice. After the sample was sonicated, the sample was centrifuged (12,000 *g*) at 4°C for 10 min and the supernatant was collected and preserved at −80°C for later analysis. Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, USA) and samples were adjusted to equal concentrations. For western blot analysis, proteins were denatured for 5 min by boiling in a loading buffer (10% sucrose, 1.2% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 0.025% bromophenol blue). Protein extracts were then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on 10% acrylamide gels and transferred to nitrocellulose membranes (Bio-Rad, Hercules, USA). Membranes were blocked with 5% non-fat milk in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween-20, and immunoblotted with primary antibody at 4°C overnight. Excess primary antibody was removed by three washes with 0.1% Tween-20. After incubation in blocking solution containing a HRP-conjugated anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, USA) with a dilution of 1 : 5000 for 1 h at room temperature, immunoreactivity was detected with enhanced chemiluminescence (ECL) using the ECL kit (Amersham Pharmacia Biotech, Buckinghamshire, England) according to the manufacturer's instructions and then visualized by autoradiography. β-Actin was used as an internal control and detected with a mouse monoclonal antibody. Densitometry calculations were performed with Sigma Scan 5 and Prism (version 4) software. Data represented the average of six independent experiments ($n = 6$).

Immunochemical staining

Astrocytes were cultured on microslips and infected with adenoviral vectors as described above. After infection with the indicated time, cells were washed with PBS and fixed in a freshly prepared 4% paraformaldehyde (Sinopharm Chemical Reagent, Shanghai, China) solution in PBS for 15 min at room temperature. Subsequently, cells were washed and incubated in PBS containing 0.1% Triton

X-100 (Sigma) for 10 min. After being washed with PBS again, the cells were incubated for 1 h in a blocking solution of PBS containing 2% non-fat milk (Bright Dairy and Food, Shanghai, China) at room temperature. Cells were then incubated in blocking solution containing primary antibody overnight at 4°C. Cells were washed and incubated in a blocking solution containing Cy3-conjugated donkey anti-mouse immunoglobulin G antibody (Jackson ImmunoResearch Laboratories) with a dilution of 1 : 600 and/or fluorescein-isothiocyanate-conjugated donkey anti-rabbit immunoglobulin G antibody with a dilution of 1 : 800 for 2 h at room temperature. After rinsing in PBS, the nuclei were stained with 4′,6′-diamidino-2-phenylindole (DAPI; Sigma) for 10 min at room temperature. Finally, immunostained cells were rinsed with PBS, cover slipped and examined with a fluorescence microscopy (Eclipse TE 2000-U, Nikon, Tokyo, Japan) equipped with a filter system or a confocal microscopy (C1 plus sci; Nikon). Microslips were randomly scanned, and six microslips were used for each group. The confocal images were captured by averaging of four laser scans and imported to Adobe Photoshop.

RNA purification and qRT-PCR

RNA purification and cDNA synthesis were performed using Trizol reagent and Superscript II (Invitrogen, Carlsbad, USA), respectively, following the manufacturers' protocols. qRT-PCR was performed using an iCycler Thermal Cycler with a multicolor real-time polymerase chain reaction (PCR) detection system (Bio-Rad). Each cDNA sample was diluted 80 folds and amplified in a 25-μl volume, using the SYBR Premix EX Taq^{TM} kit (TaKaRa, Dalian, China), with 500 nM final concentrations of each primer. The amplification cycles consisted of an initial denaturing cycle at 95°C for 10 s, followed by 40 cycles of 10 s at 95°C, 10 s at 60°C, and 20 s at 72°C. Fluorescence was quantified during the 60°C annealing step, and product formation was confirmed by means of melting curve analysis (55−94°C). Three different batches of cDNA and three independent PCRs were examined and normalized to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ($n = 3$). Relative quantification of the target gene was analyzed using the $2^{-\triangle\triangle Ct}$ method as described previously [36].

The primers shown in **Table 1** were generated by TaKaRa Company. Rat BDNF I, II, III, IV sense, and V antisense were designed according to previous report [13].

Statistical analysis

All data were expressed as the mean ± standard deviation. Statistical analysis was carried out by analysis of variance followed by Student's *t*-test or Dunnett's *t*-test. $P < 0.05$ was considered to be statistical significant.

Results

Htt552-100Q selectively reduced BDNF transcription

According to our previous study, cerebral cortical astrocytes from new-born rats were isolated and then infected with an adenoviral vector encoding htt552-100Q to generate an *in vitro* HD model. Meanwhile, non-infected cultures or cultures infected with adenoviral vector encoding htt552-18Q or Ad-null were used as controls. The htt552 protein was expressed successfully in most astrocytes with relatively high levels from 1 to 4 days post-infection. Moreover, htt552-100Q formed large aggregates in some astrocytes. This recaptured the pathological hallmark of HD [35]. Using this *in vitro* HD model, we investigated the influence of htt552 on the transcription of BDNF in astrocytes.

Table 1 Primers used in this study

Primer	GenBank No.	Sequence (5′→3′)	Product size (bp)
BDNF	NM_012513.3	TCCTGATAGTTCTGTCCATTCAGCA	95
		GGGCCATTCATTCAGGCTTC	
BDNF I		GGTGGATGAGAGTTGAAGCTTGCGA	
BDNF II		GGAGCGGAGCGTTTGGAGAGCCA	
BDNF III		CAGGAGTACATATCGGCCACCA	
BDNF IV		GGCTTTGATGAGACCGGGTTCCCT	
BDNF V		GTAGGCCAAGT TGCCT TGTCCGT	
GDNF	NM_019139.1	CAGAGGGAAAGGTCGCAGAG	114
		ATCAGTTCCTCCTTGGTTTCGTAG	
CNTF	NM_013166.1	AAGATTAGTTCAGACCTGACTGCTC	110
		AGTCGCTCTGCCTCAGTCATCTC	
GAPDH	NC_005103.2	TGCCCACCAGAACATCAT	357
		TAGCCATATTCGTTGTCGTA	

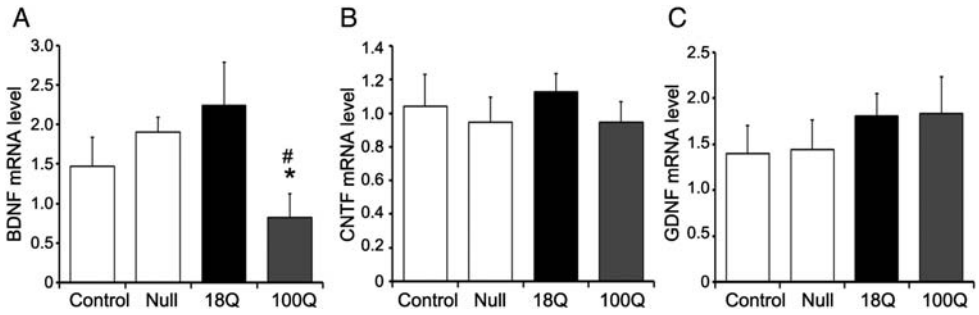

Figure 1 Influences of htt552 on the transcription of BDNF, GDNF, and CNTF Astrocytes were infected with htt552 adenoviral vectors for 48 h and total RNAs were isolated. The mRNA levels of BDNF, GDNF, and CNTF were determined by means of qRT-PCR and normalized to the levels of GAPDH mRNA: (A) BDNF, (B) CNTF, and (C) GDNF. BDNF mRNA levels were significantly reduced in astrocytes expressing htt552-100Q. $^{*}P < 0.05$ vs. Null, $^{\#}P < 0.05$ vs. 18Q. $n = 3$.

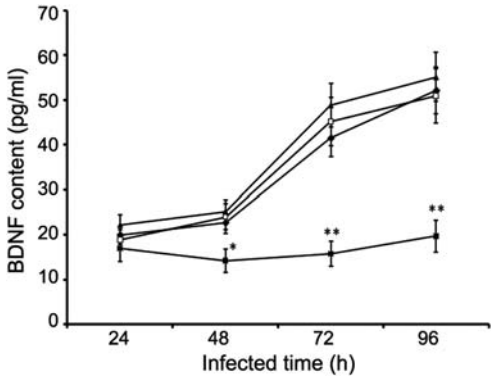

Figure 2 The production of BDNF protein from primary astrocytes was reduced by htt552-100Q Astrocytes were infected with htt552 adenoviral vectors for 24, 48, 72, and 96 h and ACM were harvested and processed for ELISA. ELISA for BDNF performed in the ACM from astrocytes expressing htt552-18Q as closed triangle, 100Q as closed square, Null as open square, and control group as closed diamond. BDNF release from cells expressing htt552-100Q was significantly lower than that in Ad-null and cells expressing htt552-18Q at 48, 72, and 96 h after infection. Values are presented as the mean ± standard deviation ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, vs. Null.

To determine whether the expression of htt552-18Q/100Q affects the gene transcription of BDNF, qRT-PCR analysis was done. Expression levels of BDNF, GDNF, and CNTF mRNAs were normalized to that of GAPDH. Compared with astrocytes expressing Ad-null or control (non-infected cells), the levels of BDNF mRNA in the astrocytes expressing htt552-18Q had no significant difference. However, the relative transcript levels of BDNF mRNA in astrocytes expressing htt552-100Q were significantly decreased ($^{*}P < 0.05$) [**Fig. 1(A)**]. The mRNA levels of CNTF and GDNF were not significantly changed in cells either expressing htt552-18Q or htt552-100Q in comparison with that of control or Ad-null cells [**Fig. 1(B,C)**]. These results suggest that htt552-100Q selectively suppresses the expression of BDNF at the transcriptional level in astrocytes.

Htt552-100Q suppressed the production of BDNF protein

To determine whether the overexpression of htt552-18Q/100Q alters the ability of astrocytes to produce BDNF, ELISA assays were performed at 24, 48, 72, and 96 h after infection. Results showed that, compared with control (non-infected cells) or Ad-null, there were no significant changes in BDNF secretion in ACM from the astrocytes infected with htt552-18Q; however, BDNF release from 100Q cells was significantly lower 48 ($^{*}P < 0.05$), 72, and 96 h ($^{**}P < 0.01$) after infection (**Fig. 2**). These results indicate that htt552-100Q reduces the expression of BDNF from astrocytes, while htt552-18Q has no significant influence on BDNF expression.

Htt552-100Q decreased the transcripts of BDNF mRNA III and mRNA IV

The rat BDNF gene contained four 5′ exons (I–IV) linked to individual promoter, which are activated in a time- and stimulus-dependent manner to produce four different transcripts and then spliced to the fifth 3′ exon (V) to produce the BDNF protein [37]. To evaluate whether the modulatory effect of htt552-100Q on BDNF gene transcription is due to the suppression of one of these promoters, we performed qRT-PCR using primers specifically recognizing each of the four BDNF exon-specific mRNAs. After 48 h infection, primary astrocytes were harvested for total RNA extraction and applied for qRT-PCR analysis. Expression levels of four different BDNF mRNA transcripts were normalized to that of GAPDH. Compared with Ad-null and control, the four transcripts of BDNF mRNAs in cells expressing htt552-18Q showed no significant change. However, significant decreases in BDNF mRNA III and mRNA IV in cells expressing 100Q were observed ($^{*}P < 0.05$) (**Fig. 3**). These results indicate that htt552-100Q inhibits the activities of promoter III and IV in BDNF gene.

Htt552-100Q aggregates altered the distribution of CBP and Sp1

To date, the mechanisms leading to the reduced expression of BDNF mRNA III and IV in HD are not fully understood. It is known that a dysfunction in the CRE activity may account for the reduced transcriptional activity of BDNF promoter III in HD [38]. It is also known that Sp1 participates in the activation on BDNF promoter IV [28,29]. Therefore, we next investigated the influences of htt552 on the transcription factors CBP and Sp1 with immunoblotting and immunofluorescence.

Results from western blot analysis showed that both CBP and Sp1 were not significantly different in astrocytes expressing with htt552-18Q or htt552-100Q compared with Ad-null and control (**Fig. 4**). However, immunofluorescence showed that htt552-100Q (shown in red) and its aggregates co-localized with CBP (shown in green) in the nucleus, which might have influence on the function of CBP. Results also showed that some Sp1 (shown in red) were sequestered into cytoplasmic aggregates formed by htt552-100Q (shown in green), which might inhibit its nuclear transport (**Fig. 5**). These observations suggest that the reduction in BDNF transcription by htt552-100Q may due to the disruption of transcriptional factors CBP and Sp1.

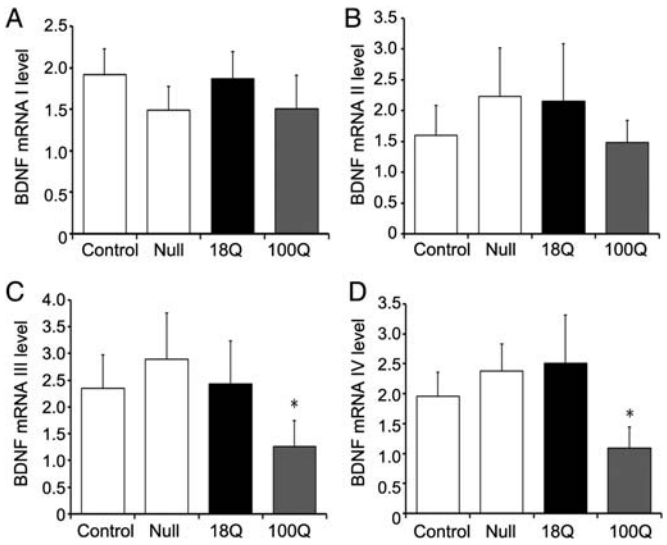

Figure 3 Influences of htt552 on the transcription of the four BDNF transcripts Astrocytes were infected with htt552 adenoviral vectors for 48 h and total RNAs were harvested. The mRNA levels of four BDNF mRNA transcripts were determined with qRT-PCR and normalized to the levels of GAPDH mRNA: (A) BDNF transcript I, (B) BDNF transcript II, (C) BDNF transcript III, and (D) BDNF transcript IV. The levels of BDNF mRNA III and IV in astrocytes expressing htt552-100Q were significantly decreased compared with control or Ad-null. $*P < 0.05$ vs. Null. $n = 3$.

Discussion

Since htt can be cleaved at certain sites by caspases, calpains, and aspartyl proteases, cleavage of wild-type htt should be a physiological event [4–9]. However, in HD, cleavage of mhtt would release N-terminal fragments with the potential for increased toxicity to neurons [14–17]. Compared with other sites, aa552 was confirmed as a preferred site for caspase cleavage *in vivo* [10]. Although there is evidence demonstrating the existence of endogenous htt552 fragment, which is localized to the perinuclear regions [39]; however, the normal function of wild-type

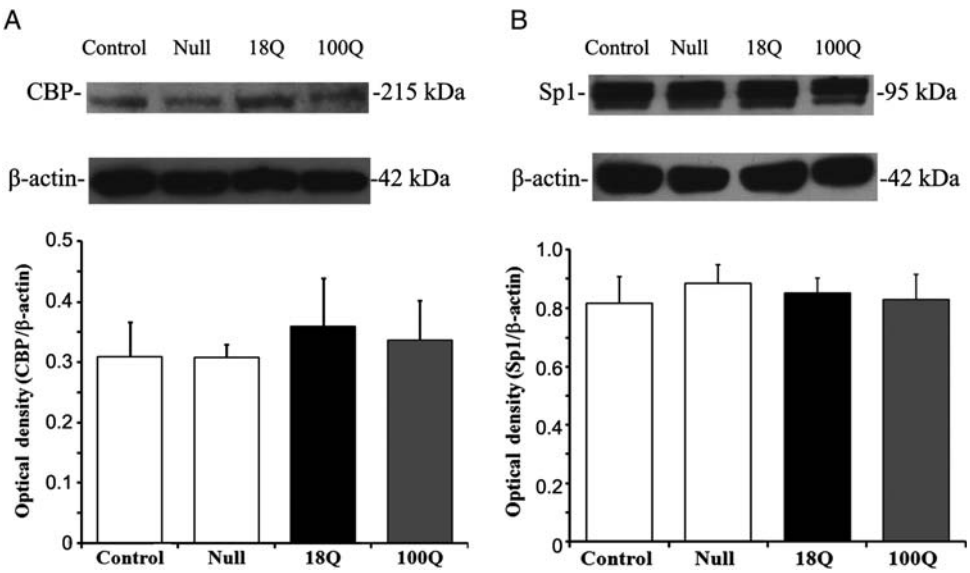

Figure 4 Effects of htt552 on CBP and Sp1 protein levels in primary astrocytes After 48-h infection of adenoviral vectors expressing htt552, astrocytes were harvested and total proteins were extracted for western blotting analysis: (A) CBP and (B) Sp1. Quantitative analysis showed that the protein levels of CBP or Sp1 in astrocytes expressing wild or mutant htt552 were not significantly different when compared with control or Null. These data were converted to the percentage of loading control (β-actin) for presentation in the bar figures ($n = 6$). Control, indicates non-infected cultures.

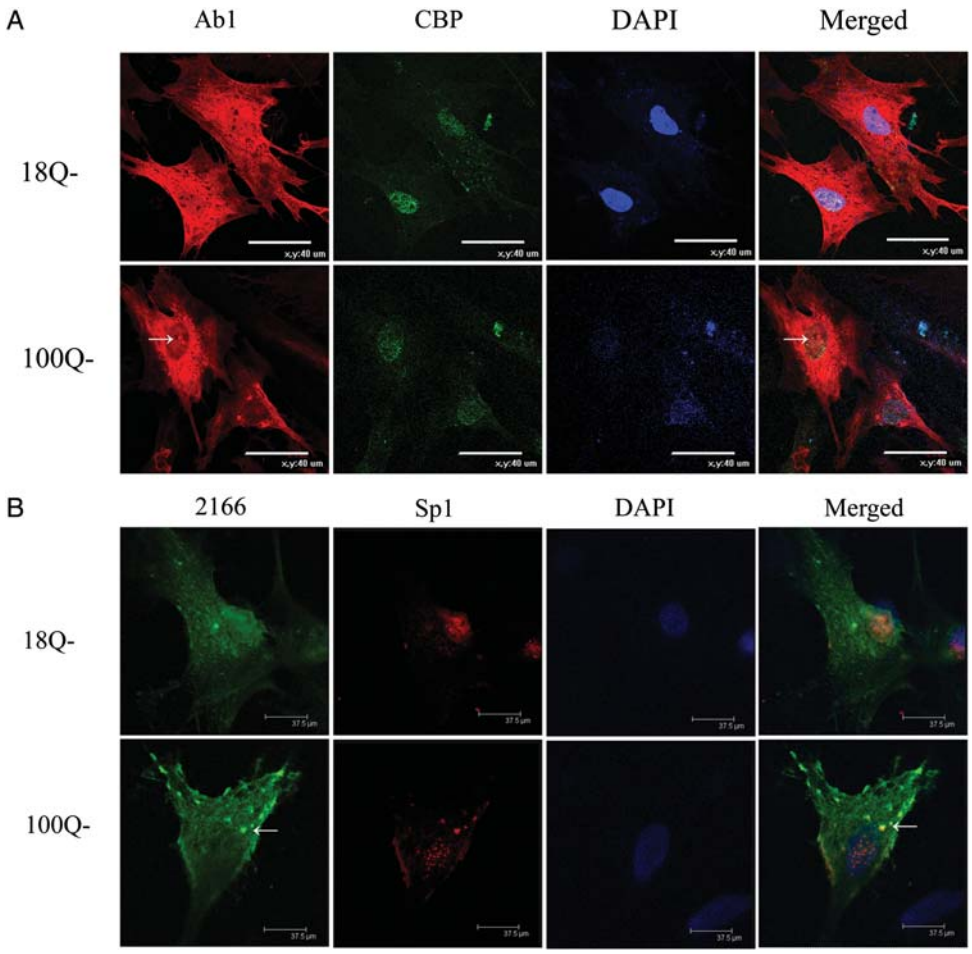

Figure 5 Aggregates formed by htt552-100Q trapped CBP or Sp1 in astrocytes Cells were visualized with confocal microscopy (×630). (A) Htt552 co-localized with CBP in the nuclei of astrocytes. Htt552 were shown in red with Ab1 antibody, while the DAPI identified the nuclei in blue. CBP was shown in green with anti-CBP antibody. Bar = 40 μm. Htt552-18Q and CBP distributed in the nucleus of infected astrocyte diffusely. Htt552-100Q formed nuclear inclusions and co-localized with CBP (arrow indicated). (B) Htt552 co-localized with Sp1 in astrocytes. Htt552 were shown in green with 2166 antibody, while the DAPI identified the cell nuclei in blue. Sp1 were shown in red with anti-Sp1 antibody. Bar = 37.5 μm. Htt552-18Q and Sp1 distributed in the cytoplasm of infected astrocytes diffusely. Htt552-100Q formed aggregates that located in the cytoplasm entrapped Sp1 and retained it in the cytosol (arrow indicated).

htt552 and pathological roles of its mutation are still unaddressed. *In vitro* studies suggested that expression of htt fragments with expanded polyQ induced apoptosis and inhibition of caspase-mediated cleavage reduces toxicity of mhtt [6,12]. In contrast, a study showed that blocking proteolysis at aa552 in the caspase-3-resistant mice did not prevent the development of neuropathological features of HD, while preventing htt cleavage at aa586 prevented the development of the HD features as observed in the YAC128 mice [40]. These findings suggested that the function of mutant htt552 was still controversial. In the present study, our findings showed that mutant htt552 reduced BDNF production at the transcriptional level in astrocytes, suggesting the possible pathological role of mutant htt552 in HD.

A large number of studies have found reduced BDNF levels in the striatum and cerebral cortex, which may underlie the mechanism of the selective neuronal degeneration in HD [41]. Further studies on the subject of neurons *in vitro* and *in vivo* indicated that the deficit in striatal BDNF in HD might be due to the reduced BDNF transcription in the cerebral cortical neurons [21]. Many observations showed that wild-type full-length htt stimulated BDNF transcription through BDNF promoter II, not BDNF promoters I, III, and IV [13]. Subsequent findings showed that the wild-type htt activated BDNF gene expression by inhibiting the RE1/NRSE within BDNF promoter II [21]. RE1-silencing transcription factor (REST), also named as neuron-restrictive silencing factor enters the nucleus, binds, and activates RE1/NRSE [42,43]. It was reported that wild-type htt could sequestrate REST into the cytoplasm, and then prevent REST from binding to its cognate cis element in BDNF gene [21]. However, in the present study, we found that

htt552-18Q had no effects on the gene transcription of BDNF in astrocytes. It indicates that htt552, a caspase-cleaved htt fragment, lose some functions because of the loss of some essential structures.

Our present study also showed that the transcripts of BDNF mRNA III and IV were decreased in astrocytes expressing htt552-100Q, which was consistent with previous results conducted in HD animal models including YAC mice and R6/2 mice [13,22,23]. The mechanisms leading to the reduced expression of BDNF mRNA III and IV in HD are related to the dysfunction of some transcription factors including CBP and Sp1 [24–26]. Previous studies demonstrated that CBP could be sequestered into mhtt aggregates, further studies showed that altered CRE-dependent gene expression might be due to the interactions of soluble mhtt with nuclear CBP, rather than the depletion of these transcription factors by nuclear inclusions [44–46]. Soluble mhtt bound more strongly to CBP than wild-type htt, possibly contributing to repression. Soluble mhtt could also interact with both the glutamine-rich activation domain and the acetyl transferase domain of CBP, which led to the reduction in the acetyltransferase activity of CBP and caused a reduction in histone acetylation [38]. In consistent with previous study, our data showed that htt552-100Q and its aggregates co-localized with CBP in the nucleus, which might have influence on the transcriptional activity of CBP.

In HD, mhtt sequesters Sp1, thus blocks its interaction with TAFII-130 and causes the reduced transcription from BDNF promoter IV [27–29]. Some findings showed that the binding of Sp1 to the specific promoters of susceptible genes was decreased in human HD brain and transgenic HD mouse brain, despite the normal protein levels and the normal to increased overall nuclear-binding activity of Sp1 [47]. Previous studies using *in vitro* binding and immunoprecipitation assays showed that htt interacts with Sp1, and polyQ expansion enhanced the interaction of mhtt with Sp1 [28,29]. Sp1 is synthesized in the cytosol and then transported into the nucleus. Our findings showed that Sp1 was sequestered into cytoplasmic aggregates formed by htt552-100Q. Mhtt552 may sequester Sp1 in the cytosol and reduce its transport into the nucleus, due to the increasing binding activity between mhtt552 and Sp1. However, the exact mechanism needs to be further studied.

According to our previous study, the percentage of astrocytes bearing cytoplasmic aggregates only reached ~15% of htt522-100Q expressing cells in the glial HD model used in the present study [35]. Therefore, besides the influence of aggregates, the soluble htt552-100Q oligomers may also contribute to the decreased transcription of BDNF. Although CBP and Sp1 can be sequestered into mhtt aggregates, altered CRE or Sp1-dependent gene expression may also be due to the interactions of soluble htt552-100Q with nuclear CBP or Sp1, which need to be further studied.

Historically, the researchers on HD have focused on the disorders in neurons, and paid little attention to the pathology of astrocytes. However, accumulating data revealed that glial cells and astrocytes, in particular, play an important role in pathology of HD. Several studies reported that mhtt decreased the expression of an astroglial glutamate transporter. The consequence of lower astrocytic glutamate uptake might accelerate excitotoxicity and thus contribute to the neuronal damage in HD [48–52]. In the present study, we demonstrated a reduction of BDNF transcription in astrocytes by mutant htt552, suggesting a novel aspect of an astrocytic defect that might cause neuronal dysfunctions in HD. Although several previous studies showed that BDNF was mainly present in neurons, and astrocytes were not the major resource of BDNF [10,51,52]; however, considering the ~10 folds more numbers of astrocytes than that of neurons, the production of BDNF from astrocytes may have profound impact on neurons in HD and other neurodegenerative disorders.

In summary, our present data demonstrated that mutant htt552 reduced BDNF transcription in astrocytes, through the entrapment of CBP and Sp1 by aggregates formed by mutant htt552. These results suggest a mechanism by which mutant htt552 affects normal function of astrocytes, thereafter might induce dysfunction of neurons in HD. Moreover, this study may provide a potential strategy for clinical treatment of HD patients.

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