Sodium Butyrate Ameliorates Histone Hypoaetylation and Neurodegenerative Phenotypes in a Mouse Model for DRPLA

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Drerontorubral-pallidoluysian atrophy (DRPLA) is a progressive neurodegenerative disease caused by polyglutamine expansion within the Atrophin-1 protein. To study the mechanism of this disease and to test potential therapeutic methods, we established Atro-118Q transgenic mice, which express in neurons a mutant human Atrophin-1 protein that contains an expanded stretch of 118 glutamines. Consistent with the results from previous studies on transgenic mice that expressed mutant Atrophin-1 with 65 glutamines, Atro-118Q mice exhibited several neurodegenerative phenotypes that are commonly seen in DRPLA patients, including ataxia, tremors, and other motor defects. Overexpression of wild-type human Atrophin-1 could not rescue the motor and survival defects in Atro-118Q mice, indicating that the mutant protein with polyglutamine expansion does not simply function in a dominant negative manner. Biochemical analysis of Atro-118Q mice revealed hypoacetylation of histone H3 in brain tissues and thus suggested that global gene repression is an underlying mechanism for neurodegeneration in this mouse model. We further show that intraperitoneal administration of sodium butyrate, a histone deacetylase inhibitor, ameliorated the histone acetylation defects, significantly improved motor performance, and extended the average life span of Atro-118Q mice. These results support the hypothesis that transcription deregulation plays an important role in the pathogenesis of polyglutamine expansion diseases and suggest that reversion of transcription repression with small molecules such as sodium butyrate is a feasible approach to treating DRPLA symptoms.

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§The abbreviations used are: DRPLA, dentatorubral-pallidoluysian atrophy; polyQ, polyglutamine; HDAC, histone deacetylase; SB, sodium butyrate; SAHA, suberoylanilide hydroxamic acid; NSE, neuron-specific enolase; PBS, phosphate-buffered saline; HD, Huntington’s disease.

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disease characterized by a number of symptoms that include ataxia, chorea, seizure, myoclonus, incoordination, and dementia (1). This disease is caused by expansion of a polyglutamine (polyQ) repeat within the Atrophin-1 protein. The glutamine stretch in Atrophin-1 ranges from 6 to 35 in normal individuals and expands from 48 to 88 in DRPLA patients (2–4). Neuropathological analyses of DRPLA patients identified widespread nuclear inclusion bodies and loss of neurons in specific brain regions such as the dentate cerebellar nucleus, red nucleus, globus pallidus, and subthalamic nucleus (1, 5–7). DRPLA and several other disorders, including Huntington’s disease (HD), spinobulbar muscular atrophy, and at least six types of spinocerebellar ataxia, constitute a family of neurodegenerative diseases that are caused by polyQ-expanded proteins (8–10).

The DRPLA causative protein Atrophin-1 is an evolutionarily conserved transcription regulator. Drosophila Atrophin (Atro) functions as a transcription corepressor, which interacts with the transcription repressors Even-skipped (Eve) and Huckebein (Hkb) during embryonic development (11). The human Atrophin-1 protein was shown to interact in vitro with ETO/MTG8, a component of nuclear receptor corepressor complexes (12), and suppress gene transcription in vivo (11). Human Atrophin-1 can also form a heterodimer with Atrophin-2, another Atrophin family protein capable of recruiting the chromatin remodeling enzyme histone deacetylase 1 (13). Interestingly, polyQ-expanded Atrophin-1 can also function as a transcription repressor, although it is less potent than the wild-type protein in a fly-based reporter assay (11). However, a separate study showed that the binding between Atrophin-1 and Atrophin-2 was enhanced by the presence of a polyQ expansion in Atrophin-1 (14). Moreover, polyQ-expanded Atrophin-1 was found to interact with transcription coactivators such as CBP and TAFII130, and inhibit the CREB-mediated gene transcription (15, 16). Thus far, the published data are not sufficient to distinguish whether the polyQ expansion of Atrophin-1 results in a dominant negative effect or a gain of function. It is generally hypothesized that deregulation of transcription is a key contributor to the pathogenesis of DRPLA and other polyQ expansion diseases (11, 17). Consistent with this idea, many changes of gene expression have been observed in mouse models of HD and DRPLA (18).

PolyQ-expanded proteins cause the deactivation of CBP, which has a histone acetyltransferase activity, and lead to cellular toxicity (16, 19). It suggests that the balance of histone acetylation is a good target for therapeutic intervention of polyQ expansion diseases (20). HDACs catalyze histone deacetylation, a reaction opposite of HAT-mediated histone acetylation, and thus induce chromatin compaction and gene repression (21–23). HDAC inhibitors, such as sodium butyrate (SB) and suberoylanilide hydroxamic acid (SAHA), have been shown to promote histone acetylation and modulate gene expression (24, 25). SB and SAHA could arrest polyQ-dependent neurodegeneration and reduce...
lethality in a Drosophila model of HD (26). They were also successfully used to ameliorate neurodegeneration in mouse models of HD and spinobulbar muscular atrophy (27–29). However, the effects of SB or SAHA on a DRPLA model have never been tested.

Previously, two independent groups had established transgenic mice that expressed mutant Atrophin-1 with an expanded stretch of 65 or 78 glutamines (30, 31). It was demonstrated that polyQ-expanded Atrophin-1 can cause behavioral and neuropathological defects in transgenic mice similar to that seen in DRPLA patients (32). Here, we used an independently generated DRPLA mouse model to further investigate the disease mechanism and examine the therapeutic potential of SB in treating DRPLA symptoms.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—Full-length human atrophin-1 cDNAs with a stretch of CAG repeats encoding 18 or 118 consecutive glutamines were cloned into the NSE transgenic vector with rat neuron-specific enolase promoter (a gift from Dr. Hanxiang Deng, Northwestern University) to construct Atro-18Q and Atro-118Q, respectively. The transgenes were linearized by NotI digestion before pronuclear injection following standard protocols (33). Toe DNA was analyzed by PCR with the following primers: TransQ-1 (5′-H11032-GCCTCAGGCTCCAACCTTCTAAG-3′) and TransQ-2 (5′-H11032-AGGCAGGTTCGGTGTCACATGG-3′). For Southern Blotting, tail DNAs were digested with EcoRV and probed with labeled fragments of the human atrophin-1 coding sequence (bp 2691–3568). Atro-118Q3 founder mice were from the mixed background of C57BL/6J and SJL/J (Jackson Laboratories). Atro-118Q3 and Atro-118Q14 mice were backcrossed to the C57BL/6J background for four to six generations before phenotypic analysis and SB treatment. The transgene in the Atro-118Q3 line could be bred to homozygosity; the attempt to generate homozygous animals for the Atro-118Q14 transgene failed because a female sterility was
associated with the hemizygotes. Atro-118Q14 mice were also backcrossed to the FVB/NJ background for four generations before crossing with Atro-18Q mice on the FVB/NJ background. The genotypes of Atro-118Q3(Tg/Tg) mice were determined by test breeding for more than 20 progenies.

Western Blotting Analysis—Brain tissues were homogenized on ice in 3 volumes of radioimmune precipitation assay buffer (1× PBS (pH 7.4), 1% Nonidet P-40, 0.5% SDS, and complete protease inhibitors (Roche Applied Science)), and incubated for 45 min. The supernatants were collected by centrifuge twice at 15,000 × g for 10 min. Protein concentrations were assessed using a BCA kit (Pierce). After running on SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes (Millipore) using 2 mM Tris, 192 mM glycine, and 20% methanol as the transfer buffer. For better detection of Atrophin-1, 0.005% SDS was added to the transfer buffer to enhance protein transfer. Western blotting was performed following a protocol described in Research Applications from Santa Cruz Biotechnology (Santa Cruz, CA), and the signals were detected using a chemiluminescent substrate (Pierce). Signal intensity of each band was calculated using the National Institutes of Health Image software (version 1.61). The primary antibodies were anti-Atrophin-1 (sc-10301), anti-c-Myc (sc-789), and anti-α-Tubulin (sc-12462) (Santa Cruz Biotechnology).

Histone acetylation was determined following a reported procedure (34). Acid extracts of brain proteins were probed with anti-acetyl-histone H3 rabbit antiserum (Upstate 07-353).

Immunohistochemistry—Brains were embedded in OCT compound and frozen in isopentane precooled in liquid nitrogen. Frozen sections (10 μm) were fixed in cold acetone. Immunostaining was done with anti-Atrophin-1 antibody (Santa Cruz Biotechnology, sc-10301) following an ABC staining procedure (Santa Cruz Biotechnology) with 3,3′-diaminobenzidine tetrahydrochloride as the substrate.

Brain Section for Nissl Staining—Mice were anesthetized and perfused with 4% paraformaldehyde in PBS (pH 7.4). Brains were post-fixed in the same fixative and processed for paraffin embedding. Coronal sections (10 μm) were done for Nissl staining with Cresyl Violet (Sigma). The somal size of neurons with obvious nucleoli was measured using a microscope (Leica DMRXA2).

Survival and Behavioral Assays—All mouse experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care & Use Committee of Institute of Developmental Biology and Molecular Medicine, Fudan University.

Rotarod performance was assessed on a rod rotating at 10 revolutions per minute (Tianhuan Instruments). Mice received six training trials from the age of 18 to 20 days. After training, four trials were given weekly to get an average score. A cutoff time of 180 s was set for each trial.

Hanging wire performance was assessed on an inverted wire (60 × 60 cm), designed according to a reported protocol (35). Mice received the same training as in the rotarod test. Two trials were given weekly with a cutoff time of 180 s.

Administration of SB—A daily dose of SB at 0.5, 1.5, or 4.5 mg/kg body weight was administrated to Atro-118Q14 mice and wild-type mice starting from the age of 4 weeks via intraperitoneal injection. Sodium butyrate (Alfa Aesar) was dissolved in PBS (pH 7.4) and made fresh daily. Control groups received PBS alone.

Statistical Analysis—Data were collected in Microsoft Excel 2000 and analyzed with Analyze-it (version 1.71). Statistical comparisons were performed using an unpaired t test (confidence interval, 99%) to calculate the p value.

RESULTS

Atro-118Q Transgenic Mice Exhibit Neurodegenerative Phenotypes—To establish a mouse model for DRPLA, we used the neuron-specific enolase (NSE) promoter to drive the expression of human atrophin-1 in neurons (Fig. 1A, also see “Experimental Procedures”). Two transgenic founders were obtained for the atrophin-1 transgene, Atro-118Q, which carries an expanded stretch of CAG repeats encoding 118 glutamines. As determined by Southern blotting (supplemental Fig. S1), one of the founders, Atro-118Q14, carried two copies of the transgene in a head-to-head orientation, whereas the other founder, Atro-118Q3, carried only one copy of the transgene. Totally, we established two hemizygous and one homozygous line from these two founders: Atro-118Q14, Atro-118Q3, and Atro-118Q3(Tg/Tg), respectively (see “Experimental Procedures”).

Both Atro-118Q14 and Atro-118Q3(Tg/Tg) mice exhibited neurodegenerative phenotypes, including feet-clasping (Fig. 1B), tremor, and ataxic gait. However, Atro-118Q3 mice did not show any of these phenotypes when compared with nontransgenic mice. Western blotting of brain protein extracts with the anti-Atrophin-1 antibody (sc-10301)
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118Q14 and Atro-118Q3(Tg/Tg) mice progressively lost the capability to coordinate and balance in the rotarod test, while Atro-118Q3 had normal rotarod performance (Fig. 1E). The hanging wire test also revealed motor defects in Atro-118Q14 and Atro-118Q3(Tg/Tg) mice (Fig. 1F) at a slightly later time point.

Protein aggregation in the nucleus, a pathological phenomenon in DRPLA patients and previous DRPLA mouse models, was also detected in Atro-118Q mice. Immunohistochemistry with the anti-Atrophin-1 antibody (sc-10301) was performed on coronal brain sections. Samples from 12-week-old Atro-118Q14 mice displayed extensive nuclear accumulation of Atrophin-1 and widespread nuclear inclusions in several brain regions (Fig. 2, A–H). Similar phenotypes were also observed in Atro-118Q3(Tg/Tg) mice at the same age, whereas aged Atro-118Q3 mice had a much weaker nuclear accumulation and nearly no inclusions (data not shown). As a control, nontransgenic mice only had a very weak nuclear immunoreactivity under the same assay conditions (Fig. 2I).

We also observed changes of neuron morphology in the dentate cerebellar nucleus, the brain region usually affected in DRPLA patients. In Atro-118Q14 mice, neurons in the dentate cerebellar nucleus displayed somal atrophy, which was absent in the control littermates (Fig. 2F). We further measured the somal area of these neurons and sorted them into ten size groups (Fig. 2K). The result showed that the average somal size of these neurons in Atro-118Q14 mice was significantly smaller than that of the nontransgenic littermates (328.6 ± 4.1 μm² for Atro-118Q14 and 429.4 ± 11.5 μm² for the control group). Similar somal atrophy of neurons in the dentate cerebellar nucleus was also observed in Atro-118Q3(Tg/Tg) mice but not in Atro-118Q3 mice (data not shown).

The Motor and Survival Defects in Atro-118Q Mice Are Not Due to a Dominant Negative Effect—The dominant inheritance of DRPLA and other polyQ expansion diseases suggests that the defects may be due to either a hyperactive, dominant negative, or neomorphic role of the mutant gene. To further understand the dominant feature of polyQ expansion, we generated Atro-18Q transgenic mice expressing human wild-type Atrophin-1 with 18 glutamines (Fig. 3A, also see "Experimental Procedures"). If the phenotypes observed in our Atro-118Q mice result from a dominant negative effect of polyQ-expanded Atrophin-1, overexpression of wild-type Atrophin-1 would be expected to suppress the abnormality caused by polyQ-expanded Atrophin-1.

Female Atro-18Q mice were crossed to male Atro-118Q14 mice, and their offspring were classified into four groups based on their genotypes: Atro-118Q/Atro-18Q, Atro-118Q, Atro-18Q, and nontransgenic mice. Western blotting for Atrophin-1 expression showed that expression levels of wild-type Atrophin-1 are much higher than Atrophin-1(118Q) in Atro-118Q/Atro-18Q mice (Fig. 3A).

Rotarod and hanging wire tests were used to assess the motor performance of these four groups. At the age of 5 and 9 weeks, Atro-118Q/Atro-18Q mice were severely impaired in rotarod tests, and no statistical difference was seen between the performance of Atro-118Q/Atro-18Q and Atro-118Q mice (Fig. 3B). The hanging wire test also indicated no statistical difference between the performance of Atro-118Q/Atro-18Q and Atro-118Q mice (Fig. 3C). Atro-18Q mice showed normal performance similar to nontransgenic mice in the rotarod and the hanging wire test (data not shown). The average life span of Atro-118Q/Atro-18Q and Atro-118Q mice also showed no significant difference (Fig. 3D). The inability of wild-type Atrophin-1 to rescue the motor or survival defects in Atro-118Q mice suggests that the defects in Atro-118Q mice are not likely due to a dominant negative effect of the polyQ expanded transgene. The effects of Atro-118Q transgene may be due to a neomorphic function, or alternatively, hyperactivation of the normal transcription repressor function of Atrophin-1.
Sodium Butyrate Treatment Ameliorates the Motor and Survival Defects in Atro-118Q Mice—Several HDAC inhibitors, such as SB and SAHA, have shown therapeutic effects in mouse models of HD and spinobulbar muscular atrophy (27–29). SB is less toxic than SAHA and more soluble in aqueous solution. SB administration in Atro-118Q mice provides an effective approach to test the effects of HDAC inhibitors on DRPLA. A daily dose of SB at 0.5, 1.5, or 4.5 mg/kg body weight was administrated to Atro-118Q14 mice starting from the age of 4 weeks via intraperitoneal injection. SB treatment at a dose of 0.5 or 1.5 mg/kg did not cause obvious adverse effects during the first week, but mice treated with 4.5 mg/kg SB failed to gain weight. Therefore, only the two lower doses were chosen for subsequent experiments.

The effects of SB treatment were assessed by motor and survival assays. The rotarod performance of Atro-118Q mice was significantly improved after SB treatment at both doses, although the effect of the higher dose is more obvious (Fig. 4A). The high dose at 1.5 mg/kg significantly improved the performance from 5 to 9 weeks of age. The low dose at 0.5 mg/kg also improved rotarod performance, but only from 5 to 6 weeks of age. The hanging wire test also indicated the ameliorative effects of SB treatment on Atro-118Q mice from the age of 7 to 9 weeks (Fig. 4B). Therefore, SB treatment delayed the onset of motor impairments in Atro-118Q mice. We also tested the effects of SB treatment on nontransgenic mice. The high dose at 1.5 mg/kg did not change the performance of nontransgenic mice when compared with PBS-treated mice (data not shown).

SB treatment not only improved the performance of Atro-118Q mice in the behavioral assays but also extended their average life span. SB treatment (1.5 mg/kg) starting from 4 weeks of age increased the average life span of Atro-118Q mice by 13.7% over that of PBS-treated mice (Fig. 4C).

As described above (Fig. 4, A and B), early-onset SB treatment (from 4 weeks old) significantly improved the motor performance of Atro-118Q mice from 5 to 9 weeks old. However, SB-treated mice had similar levels of motor impairments as PBS-treated mice after 12 weeks of age. We thus asked whether late-onset SB treatment starting from 12 weeks old, when the motor impairments are fully developed, could also suppress or reverse the motor defects in Atro-118Q mice. Our results showed that late-onset SB treatment (1.5 mg/kg) could not improve motor performance in Atro-118Q mice (data not shown). However, late-onset treatment had a beneficial effect on the survival of Atro-118Q mice, as it did extend the average life span of these mice, which was 21.5% longer than that of PBS-treated mice (Fig. 4D). These results indicate that late-onset SB treatment may be effective in antagonizing polyQ toxicity after the development of behavioral impairments.

Comparing the somal size of neurons in dentate cerebellar nucleus, we did not find obvious difference between SB-treated (1.5 mg/kg) and PBS-treated Atro-118Q14 mice (328.2 ± 5.2 μm² for SB-treated group and 325.3 ± 8.5 μm² for PBS-treated group).

SB Treatment Suppresses Hypoacetylation of Histone H3 in Atro-118Q Mice—If the ameliorative effects of SB treatment result from its ability to inhibit HDACs, we expect that the SB treatment should have a direct effect on levels of histone acetylation. We tested this hypothesis by examining the acetylation of histone H3 using an antibody specific to acetylated histone H3. We found that histone H3 was hypoacetylated in Atro-118Q4 and Atro-118Q3(Tg/Tg) mice but not in Atro-118Q3 mice, and the acetylation level was inversely correlated with the phenotype severity in Atro-118 mice (Fig. 5, A and B). The acetylation level of histone H3 in Atro-118Q4 mice was ~44% of that in nontransgenic mice. This histone hypoacetylation could be suppressed by SB treatment as the acetylation level was recovered to ~93% by SB treatment (1.5 mg/kg) from the age of 8 to 12 weeks (Fig. 5, C and D).

It is also possible that SB treatment reduced the expression level of Atro-118Q transgene, which subsequently ameliorated the phenotypes in transgenic mice. This possibility was investigated with several assays. Western blotting analysis showed that SB treatment (1.5 mg/kg) from 4 to 12 weeks old did not cause any changes in the expression level of Atro-118Q transgene or the level of endogenous Atrophin-1 (supplemental Fig. S2A). The same treatment also did not alter the nuclear accumulation of Atrophin-1 and the nuclear inclusions in the cortex, cerebellum, or striatum (supplemental Fig. S2B and data not shown). Quantification of nuclear inclusions in the cortex revealed no obvious difference between SB-treated and PBS-treated Atro-118Q mice (supplemental Fig. S2C). Therefore, our results strongly suggest that inhibition of HDACs by SB and a global increase of histone acetylation are antagonistic to polyQ-induced toxicity.

DISCUSSION

Several key clinical features relevant to DRPLA can be quickly evaluated in Atro-118Q transgenic mice by simple behavioral tests. Atro-118Q mice exhibited ataxia and incoordination in the rotarod test,
which may correlate with dysfunction of the cerebellum (35–37). In the hanging wire test, Atro-118Q mice showed tremor and myoclonus, indicating a neuromuscular abnormality (35, 38). These two behavioral tests provide a noninvasive and quantitative readout of motor defects in Atro-118Q mice at different ages, thereby facilitating further research on DRPLA pathogenesis and therapy.

Neuropathological analysis showed that widespread nuclear inclusions were observed in various brain regions of Atro-118Q mice, consistent with the pathological feature in DRPLA patients. Moreover, selective lesions in specific brain regions, such as the dentate cerebellar nucleus, are another pathological feature of DRPLA. In Atro-118Q mice, we found an observable somal atrophy in the neurons of the dentate cerebellar nucleus, consistent with the degeneration of the dentate cerebellar nucleus that was found in DRPLA patients (39, 40). Autopsy of some DRPLA cases also showed loss of neurons in the dentate cerebellar nucleus (41). However, no obvious change of neuron density was seen even in antemortem Atro-118Q mice (data not shown). This pathological discrepancy between DRPLA patients and Atro-118Q mice suggests that it may be a long term process from a detectable change of neuron morphology to neuron loss. The shorter survival of Atro-118Q suggests that it may be a long term process from a detectable change of neuron morphology to neuron loss. The quicker disease progress might limit the effect of SB treatment on the neuronal atrophy of Atro-118Q mice.

Three transgenic mouse lines were established with different expression levels of the Atro-118Q transgene. Phenotypic analysis revealed a dosage effect of the Atro-118Q transgene on the severity of the neurodegenerative phenotypes. A single copy of the transgene in Atro-118Q3 mice did not cause obvious motor and survival defects, whereas double copies of the transgene in Atro-118Q3(Tg/Tg) and Atro-118Q14 mice increased transgene expression and caused motor impairments and early death. In pathological analyses, the brains of Atro-118Q3 mice showed weak nuclear accumulation of Atrophin-1 and nearly no nuclear inclusions. These results indicate that a certain level of polyQ-expanded Atrophin-1 can be tolerated without inducing obvious neurodegenerative phenotypes, at least during the time span used in this study. Previous studies have shown that misfolded proteins with polyQ expansion can be eliminated by the molecular chaperones and the proteasome complex in neurons (42, 43). Thus, the Atro-118Q3 mouse line provides a good model to study how neurons eliminate polyQ-expanded proteins and protect themselves against polyQ toxicity.

We showed that wild-type Atrophin-1 does not modify the motor and survival defects in Atro-118Q mice. This result suggests that these defects may not be caused by a dominant negative effect of mutant Atrophin-1. The mutant protein is also not likely to be hyperactive, because overexpression of wild-type Atrophin-1 in Atro-118Q mice did not result in neurodegenerative phenotypes. We also found that Atro-18Q transgene did not cause observable changes of histone H3 acetylation (data not shown). Thus, polyQ-expanded Atrophin-1 is most likely to play a neomorphic role in the DRPLA animal model. Recent studies have shown that proteins with expanded polyQ tract had abnormal interactions with several key components of the transcription complex, including p53, CBP, TBP, Sp1, and TAFII130 (15, 16, 44–47). These abnormal interactions may disturb the transcription machinery and cause transcription deregulation in polyQ expansion diseases (11, 48).

The notion of transcription deregulation in DRPLA is further supported by the fact that several transcription regulatory proteins such as CBP, which are sequestered into the nuclear inclusions formed by polyQ-expanded proteins (16, 44, 45), had intrinsic histone acetyltransferase activity (19, 49). An imbalance between HDACs and histone acetyltransferases may change the level of histone acetylation and lead to transcription deregulation in polyQ expansion diseases (20). Here, we show hypoacetylation of histone H3 in Atro-118Q mice, and the level of histone hypoacetylation correlated with the phenotype severity in different transgenic lines. Daily intraperitoneal administration of SB, a HDAC inhibitor, successfully ameliorated both histone hypoacetylation and the neurodegenerative phenotypes in Atro-118Q mice. These results strengthen the correlation between transcription deregulation and the polyQ-induced neurodegeneration in DRPLA.

The therapeutic effect of SB in our DRPLA mouse model gives a clinical prospect of HDAC inhibitors in the therapy of DRPLA. Our study shows that SB treatment is more effective when started earlier in the course of disease progression, suggesting that transcription deregulation is an early event in DRPLA pathogenesis.

It has been shown that SB treatment had effects on the neuronal atrophy in mouse models of HD (27) and spinobulbar muscular atrophy (28). SB treatment did not obviously ameliorate the neuronal atrophy of dentate cerebellar nucleus in our DRPLA mice. Our DRPLA mouse model showed severe neurodegenerative phenotypes with early-onset. The quicker disease progress might limit the effect of SB treatment on some long term pathogenic changes, such as neuronal atrophy. Alternatively, the difference in neuronal atrophy change may reflect the unique characters of the poly-Q expansion diseases and their mouse models.

The gene expression changes caused by polyQ-induced transcription deregulation have been shown in microarray analysis of a DRPLA.
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mouse model (18). These gene expression changes may have different sensitivities to SB treatment, as studies in transformed cells found that only a small number of genes (~2%) showed changes in expression after the treatment with HDAC inhibitors (50). Therefore, it is possible that SB can only rescue a part but not all of the gene expression changes in DRPLA mice, and this incomplete amelioration of gene expression may result in the progressive loss of therapeutic potency in SB treatment. Thus, the dose and duration of SB treatment may need to be further optimized to obtain more therapeutic potency and less adverse effects. SB has also been shown to prevent oxidative neuronal death by activating Sp1-dependent gene expression (51), suggesting other possibilities may also exist in explaining the improvement of neurodegenerative phenotypes in DRPLA mice after SB treatment.

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