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Selenium Nanoparticles as an Efficient Nanomedicine for the Therapy of Huntington's Disease

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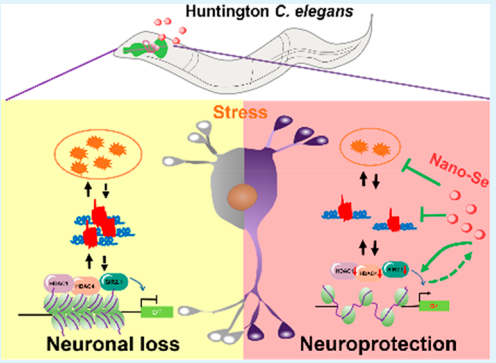
Supporting Information

ABSTRACT: Huntington's disease (HD) is an incurable disease with progressive loss of neural function, which is influenced by epigenetic, oxidative stress, metabolic, and nutritional factors. Targeting inhibition of huntingtin protein aggregation is a strategy for HD therapy, but the efficacy is unsatisfactory. Studies found that selenium (Se) levels in the brain are insufficient for HD disease individuals, while improvement in Se homeostasis in the brain may attenuate neuronal loss and dysfunction. In this study, we applied selenium nanoparticles (NPs) (Nano-Se) for the HD disease therapy by regulating HD-related neurodegeneration and cognitive decline based on transgenic HD models of *Caenorhabditis elegans* (*C. elegans*). At low dosages, Nano-Se NPs significantly reduced neuronal death, relieved behavioral dysfunction, and protected *C. elegans* from damages in stress conditions. The molecular mechanism further revealed that Nano-Se attenuated oxidative stress, inhibited the aggregation of huntingtin proteins, and downregulated the expression of histone deacetylase family members at mRNA levels. The results suggested that Nano-Se has great potential for Huntington's disease therapy. In conclusion, the mechanism about how Nano-Se NPs protect from damages in stress conditions and how they repair neural functions will benefit HD disease therapy. This study will also guide rational design of Nano-Se NPs or other selenium compounds to improve HD therapy in the future.



KEYWORDS: *Huntington's disease, selenium nanoparticles, behavioral dysfunction, oxidative stress, huntingtin protein, histone deacetylase*

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant and inherited neurodegenerative disease that causes cell death in the brain and exhibits motor, cognitive, and psychiatric disorders for the adult HD patients. It is caused by an expanded CAG trinucleotide repeat (of variable length), the gene that encodes the huntingtin protein (HTT).[1] HD is a complex multifactorial disorder converging to epigenetic factors and oxidative stress.[2,3] There is a rapidly growing body of evidence linking epigenetic alterations to the development of HD.[4] Transcriptional dysregulation is an early event and an important pathological mechanism linking nutritional availability to cellular processes in HD. Aggregates of mutant HTT may cause significantly decreased acetylation of histone that linked to neuronal damage and loss in HD.[5] The acetylation of histone is thought to play a critical role in cognitive functions such as learning and memory.[4] Oxidative stress also plays a significant role in the development and progression of HD. Cumulative oxidative stress may damage cellular structures and impair the DNA repair system and induce mitochondrial dysfunction, which have been regarded as key factors in the development of neurodegenerative disorders.[6] Studies from post-mortem brains of HD patients have demonstrated increased oxidative damage.[7] Currently, there is no effective therapy to cure or stop the progression of HD.[1,8] Most HD treatments in clinical trials have focused on targeting HTT including AAV-delivered RNAi-based therapies, CRISPR-based genome editing, HD-SNP targeting, and so forth. However, these therapeutic approaches show unsatisfactory efficacy with limitations including long-term interference in genes, off-target cleavage, and potentially adverse effects on safety.[2]

As an essential trace element for animals and human being, selenium (Se), is critical for the physiological functions of the brain with neuroprotective effects. Selenoproteins are found to play roles in the regulation of neurodegenerative disorders

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such as HD.[9,10] Observational epidemiological studies have shown that Se benefits neurological function and cognitive performance.[9] Insufficient levels of selenium in the brain and the disruption of selenium homeostasis will cause detriment to brain function and may exacerbate neuronal loss and dysfunction.[11,12] Importantly, recent studies have shown the protective role of Se in HD. For example, sodium selenite could decrease mutant huntingtin aggregation and oxidized glutathione levels in the brain of HD mice.[9] Despite neuroprotective effects, Se has a narrow window between its beneficial and toxic effects.[13,14] Biological activities of Se are influenced by its chemical form.[15,16] Different selenium compounds can be metabolized through distinct pathways.[3,15,16] Recently, selenium nanoparticles (Nano-Se) have been found to be low toxic with acceptable bioavailability and high efficiency in preventing oxidative damage compared to other forms of Se.[17−20] Nano-Se can efficiently scavenge free radicals compared to Na_2SeO_3 at a concentration below 0.5 mM.[20] Nano-Se also exhibits lower toxicity than methylselenocysteine due to less effect on compromising selenoenzymes.[18] It is still unknown whether Nano-Se performs well in the treatment of HD due to the properties. Therefore, we proposed to use Nano-Se for the therapy of HD. Moreover, considering the safety of this nanomedicine, it is necessary to confirm a dosage window for the application of Nano-Se or other selenium compounds during the HD therapy.

Caenorhabditis elegans (*C. elegans*) is one classical model animal to study structures and functions of nervous system. Its brain contains only 302 neurons, and the neuronal lineage has been already mapped, which serves as an effective model to explore physiological functions of neurological systems as well as pathological mechanism for neurological diseases.[21,22] As a dietary supplementation, the accumulation of selenium in the worms mainly lies on the intake of selenium according to the introduced Nano-Se in the food because the nematode growth medium (NGM) does not contain selenium.

Herein, wild-type and transgenic HA759 and AM141 *C. elegans* were used to evaluate therapeutic efficacy of Nano-Se for HD disease.[21,23] We first prepared and characterized Nano-Se and exposed them to *C. elegans* of the wild-type and transgenic types under normal and MeHg- or juglone-stressed conditions. We then evaluated the influence of Nano-Se on worms' survival rate under both conditions. Furthermore, we used chemotaxis behavior assays to explore protective effects of Nano-Se on the survival of ASH and physiological functions of sensory response in transgenic *C. elegans* strain HA759. Chemosensory behavior and mechanical sensory behavior were evaluated at the phenotype and gene levels. Moreover, we studied whether Nano-Se decreased mutant huntingtin aggregates burden and oxidative stress in *C. elegans* strain AM141 and revealed molecular mechanism at epigenetic levels. Finally, we correlated protective effects of Nano-Se with their antioxidative ability after their intake *in vivo*.

EXPERIMENTAL SECTION

Preparation and Characterization of Nano-Se. Nano-Se was synthesized as in a previous publication.[24] In brief, 10 mM sodium selenite was incubated with 10 mM GSH solution containing BSA in 1:4 (v/v). Sodium hydroxide was added to adjust the pH of the mixture. At pH 7.1, Nano-Se and oxidized glutathione (GSSG) formed immediately. The red solution was dialyzed in distilled water for 3 days to separate GSSG from the Nano-Se. The Nano-Se dispersion was finally stored at 4 °C for following studies. Transmission electron microscope (TEM), dynamic light scattering (DLS), and Zetasizer were used to characterize the morphology, size, and the surface charges (zeta potentials) of Nano-Se. Moreover, we used synchrotron radiation X-ray absorption near edge spectroscopy (XANES) to characterize the chemical species of Se in the Nano-Se sample. Se K-edge XANES was collected at BL-14W1A in Shanghai Synchrotron Radiation Facility (SSRF). Detailed procedures for data collection and analysis referred to our previous work.[25,26]

In brief, the reference samples including Se foil, Na_2SeO_3, and Na_2SeO_3 were smeared evenly onto a polycarbonate film and transmission mode was used to collect the XANES data. Moreover, the Nano-Se dispersion at 5 mg/mL was put into a transparent container, and the XANES data were collected under fluorescence mode. XANES data were then normalized by IFEFFIT Athena software (CARS, the Consortium for Advanced Radiation Sources at University of Chicago). Finally, the least-squares linear combination fitting was done to calculate the chemical composition of Se in the Nano-Se.

Reagents and Worm Strains. The membrane-permeable nonfluorescent dye 2,7-dichlorodihydrofluorescein diacetate (H_2-DCF-DA) can be applied to determine the ROS level.[44] The compound of 5-hydroxy-1,4-naphthoquinone (Juglone, Sigma-Aldrich) can generate reactive oxygen species as an inducer for oxidative stress. The worms were anesthetized by 1% levamisole (w/w) before the observation by fluorescence microscope. Nematode wide-type strain (N_2) and HA759 {rtIs11[osm-10p::GFP+osm-10p::HtnQ150+Dpy-20(+)]} and AM141 {rmIs133[unc-54p::Q40::YFP]} were purchased from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN). Unless otherwise noted, the worms were maintained under standard conditions on the nematode growth medium (NGM) and seeded with *Escherichia coli* strain OP50. Age-synchronous populations were obtained as previously described.[44] Because the toxicity of selenium increased with the elevated temperature at 25 °C on upon exposure, we performed all studies at 20 °C.[45]

Survival Rate and Growth. Synchronized L1 worms were exposed to Nano-Se at different concentrations for 72 h. Then, the survival rate and growth rate of worms were assessed. At least 30 worms for each group was observed and assessed.

Neurodegeneration Analysis. Synchronized L1 HA759 worms were grown in a NGM plate in the presence of 0, 0.02, 0.2, and 2 μM Nano-Se for 3 days. To assess ASH neuronal viability, HA759 strain worms were counted to calculate the fraction of live ASH neurons per group.[46] Briefly, the normal individual was scored if both ASH neurons were present without malformities such as distention, apoptotic swelling, and separation of soma as well as loss of fluorescence. At least 30 nematodes were randomly selected and scored for each tested group.

Chemosensory Behavior Assay. Synchronized L1 HA759 worms were grown in a NGM plate in the presence of 0, 0.02, 0.2, and 2 μM Nano-Se for 3 days. The chemosensory assay was performed as previously described.[47] Prior to the chemosensory assay, young adult stage worms were washed three times with S-basal buffer to remove bacteria. NGM plates were divided into two identical areas (A and B). Glycerol was spread along region A and the midline of the plate. When glycerol penetrated into chemotaxis agar, 1% butanedione (2 μL) and 200 mM NaN_3 (2 μL) were spotted on the A side of the plate, approximately 1 cm distant from the edge. After placed on B-region, the worms are attracted by butanedione, approached A region, and then were paralyzed by NaN_3 for 90 min. Afterward, the number of nematodes in areas A and B was counted, and the chemosensory index was calculated as B/(A + B).

Touch Tests. After L1 HA759 worms were synchronized, the sensitivity to mechanical sensations was observed and scored after we touched the tails of nematodes 10 times and analyzed as the ratio of the response. When a normal worm is moving forward and is tapped on the head, it will halt forward locomotion, initiating backing.[28] Worms with such behavior were scored as normal. When the head was tapped and the body paused, the continued forward of worms displays a backing deficit. In this case, the movement was scored as

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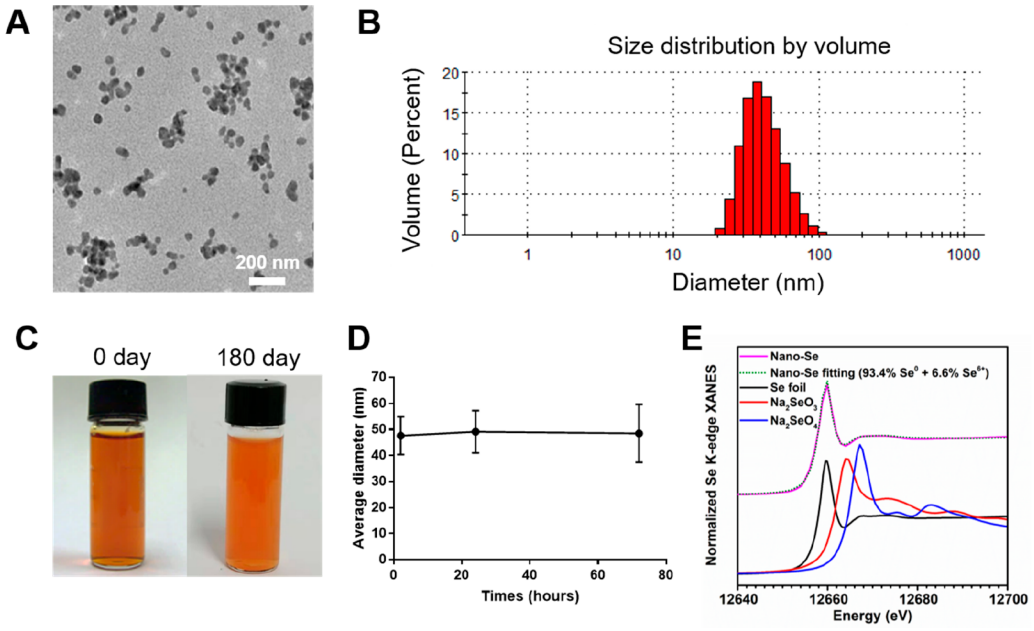

Figure 1. Characterization of Nano-Se. (A) TEM image of Nano-Se. (B) Size distribution of Nano-Se as determined by dynamic light scattering (DLS). (C, D) Stability of Nano-Se in the aqueous solution during the storage for 180 days. The photographs for the dispersibility at 0 and 180 days (C). Average diameter of Nano-Se at 0, 24, and 72 h postpreparation of dispersion (D). (E) Chemical speciation of Se for Nano-Se solution as determined by Se K-edge XANES. With respect to the Nano-Se sample, the least-squares fitting of XANES was performed based on the spectra of the reference samples including Se foil, Na_2SeO_3, and Na_2SeO_4.

backing deficit/failure. Under mechanical touch, the ratio of the response time to the touch times (per 10 times) was scored.

Huntingtin Protein Aggregates Measurement. After synchronization, AM141 worms expressing a muscle-specific polyglutamine (Q40)::YFP were grown in a NGM plate in the presence of 0, 0.02, 0.2, and 2 μM Nano-Se for 3 days from the L1 onward. Levamisole (1%) was used to anesthetize the worms before the observation. The expressed polyQ aggregates labeling with green fluorescent protein (GFP) was used to monitor PolyQ40::YFP aggregates in muscle cells. In each group, at least 30 worms were counted under a fluorescence microscope.

ROS Level Measurement. Worms were pretreated with 2 μM Nano-Se or not for 3 days. They were then exposed to 300 mM Juglone for 1 h to induce oxidative stress. Next, the worms were homogenized by sonication and centrifugation. The supernatants were transferred to a 96-well plate containing 25 mM H_2-DCF-DA in the dark. Fluorescence intensity was measured with an excitation at 488 nm and an emission at 520 nm after incubation with H_2-DCF-DA. Fluorescence intensities were normalized to the control at different time points. The ROS level could be described as related intensity of probe fluorescence by dividing that of control. At least 30 nematodes were examined per test.

Stress Resistance Assays. For Juglone stress resistance assay, Juglone was dissolved to a final concentration of 300 mM in a solution of 0.23% (v/v) ethanol in M9.[28] Prior to Juglone stress resistance assay, the wild-type N_2 worms were pretreated with 2 μM Nano-Se or not for 3 days and then were transferred to fresh NGM plates with Juglone for 8 h. Finally, the number of dead worms was counted by touch-provoked movement. Prior to MeHg stress resistance assay, the wild-type N_2 worms were pretreated with 2 μM Nano-Se or not for 2 days and then transferred to S medium containing *E. coli* OP50 bacteria and MeHg (40 μM) for 36 h. At last, the number of dead worms was counted by touch-provoked movement (Figure S1).

mRNA Level Determined by Quantitative PCR. In terms of the *nlp-3*, *qui-1* quantitative PCR test, HA759 worms pretreated with Nano-Se or not were exposed to butanedione for 90 min and collected after 12 h. In terms of the *hdac-1*, *hdac-2*, *hdac-3*, *hdac-4*, *hdac-6*,*hdac-10*, and *sir2.1* quantitative PCR test, N_2 and HA759 worms that were pretreated with 2 μM Nano-Se or not for 2 days were collected for measurement. The total mRNA in all of the groups was extracted by using TRIZOL (Invitrogen) and reverse transcribed. Quantitative real-time PCR was performed using a Bio-Rad detection system. The transcript quantity was normalized to that of the gene of actin. The primers are shown in Table S1.

Determination of Selenium *in Vivo*. To quantify the intake of selenium by the worms, we used inductively coupled plasma mass spectroscopy (ICP-MS) to determine the content of selenium accumulated in ~20 nematodes. At first, 2 μM Nano-Se was exposed to the L1 stage *C. elegans* for 3 days. To remove bacteria outside of the worm, young adult worms were rinsed three times with S-basal buffer. Then, the nematodes were broken up by sonication, and then the supernatant was collected for ICP-MS analysis. The total protein mass in 20 worms was determined by BCA. A blank without worms was used to measure Se content at background levels. Selenium isotopes of ^{77}Se and ^{78}Se were quantified in triplicate.

Statistical Analysis. Data were described as mean value and standard errors which relied on the averaging results obtained. A significant difference in different populations was determined by using one-way ANOVA and LSD *post hoc* test. Except for a specific statement, all of the experiments were repeated three times. Probability levels below 0.01 represent significant and very significant differences.

RESULTS AND DISCUSSION

Nano-Se NPs Protecting *C. elegans* Survival Under Neurotoxic Stress. Nano-Se nanoparticles used in the study were synthesized as in previous work,[24] and the morphology and the mean size of Nano-Se were characterized by a transmission electron microscope (TEM) (Figure 1A) and dynamic light scattering (DLS). The mean diameter of Nano-Se was 48 ± 4.3 nm. In the aqueous solution, the zeta potential of Nano-Se was −22.3 ± 2.6 mV, which suggested the surface charge of Nano-Se was negative and the nanoparticles have a good dispersity in the solution. The stable dispersion solution of Nano-Se exhibited a brown color and remained clear without sediments even after 180 days storage (Figure 1C).

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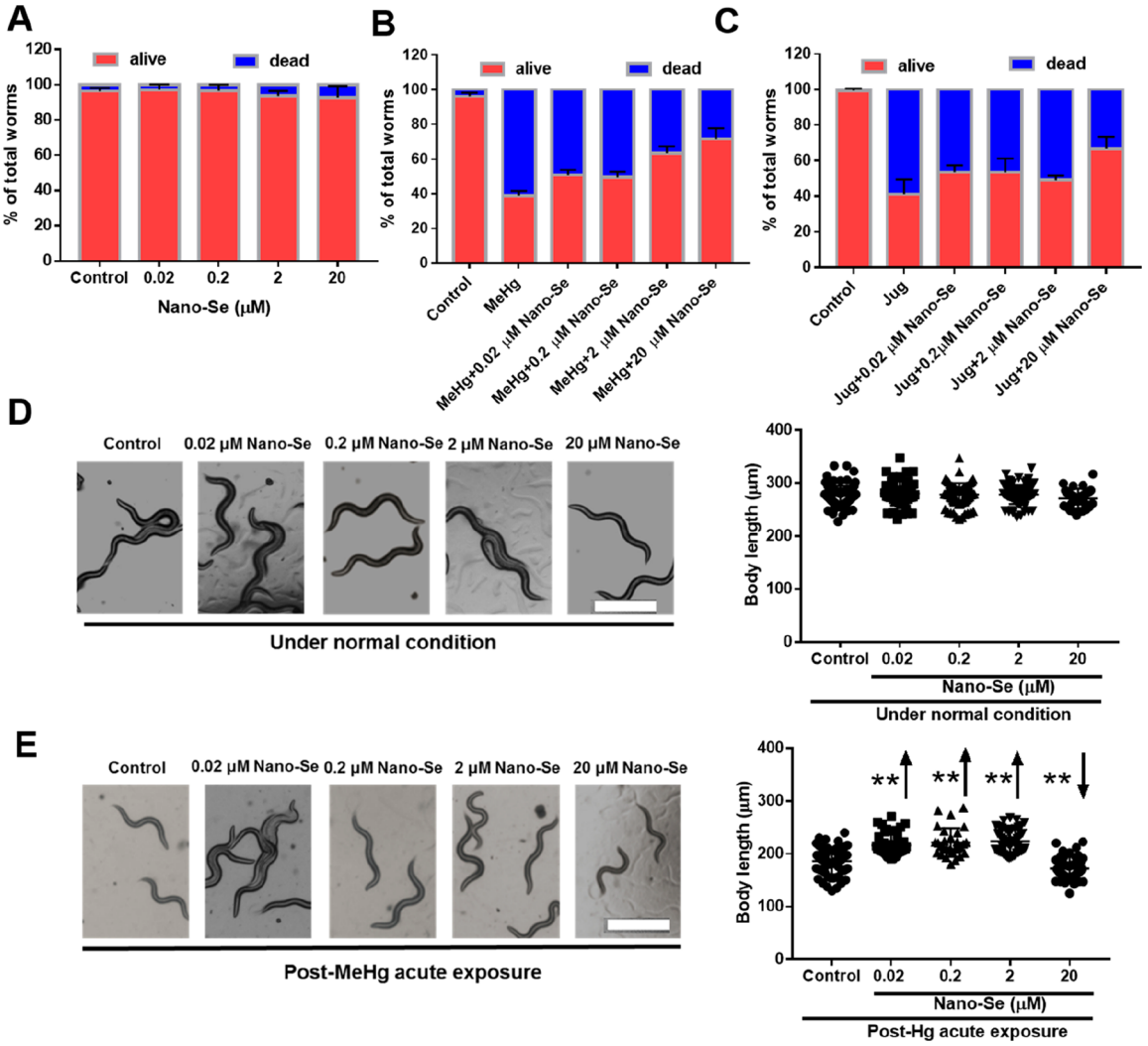

Figure 2. Influence of Nano-Se treatment on survival and body growth of *C. elegans* under normal and stressed situations. (A−C) Effects of Nano-Se on worms' survival rate under normal, MeHg, and juglone stressed situations. (D, E) Effects of Nano-Se on the body growth of worms under normal and MeHg-stressed situations. Data represent the mean value ± standard errors. The double asterisks (**) indicate a very significant difference ($p < 0.01$) between control and the Nano-Se treated worms. Scale bar represents 500 μm.

Moreover, DLS measurements also show that the average size of Nano-Se did not change at 0, 24, and 72 h postpreparation (Figure 1D), which suggested the stable condition. In addition, XANES is powerful to reveal electronic structures of elements that are capable of characterizing the chemical forms of interested elements in the liquid and solid phase as well as chemical information for the bulk sample or the surface region.[32] Herein, Se K-edge XANES spectra were collected for reference samples and Nano-Se. The fitting XANES result indicated that Nano-Se includes two compositions: 93.4% Se^0 and 6.6% Se^{6+} (Figure 1E). It meant that most Se existed as the reduced state, and a small part of Se was the oxidative state (+6) which may reside on the surface of nanoparticles. XANES results thus show Nano-Se mainly exists as the elemental Se as the major chemical form for their application.

Next, we used both survival rate and the body length to describe the protective effects of Nano-Se on the viabilities and the growth of *C elegans*, respectively. We found that Nano-Se under 20 μM did not induce an obvious toxic effect on *C. elegans* under normal situations (Figure 2A,D). Moreover, we explored the appropriate dosage of Nano-Se to afford protection under neurotoxic stress. Juglone and methylmercury (MeHg) can induce oxidative stress and lead to neurodegenerative disorders, which were adopted in this study.[27,44] Under 20 μM, Nano-Se played a dosage-dependent protective effect on the viability after the exposure to both stress stimuli (Figure 2B,C). However, Nano-Se under 2 μM played the protective effects on the growth of the worms when they are exposed to the same stress (Figure 2E). Selenium has a narrow window for the exposure dosages between its beneficial and toxic effects as a micronutrient. Its concentration is one of the critical determinants for the therapeutic efficacy. Therefore, the dosage threshold is necessary to be confirmed since the selenium compounds at high dosages will give rise to potential toxicity to neurons.[13] The threshold values vary with two evaluation modes, and the parameter of body length was more sensitive than the viability under the stress stimuli. In conclusion, according to the viability and body length, Nano-Se below 2 μM produced significant protection to *C. elegans* after their exposure to neurological stress such as Juglone and MeHg. In the following studies, the concentrations of Nano-Se were thus set as 0.02, 0.2, and 2 μM.

Protective Effects of Nano-Se on ASH Neuronal Survival. The transgenic Huntington *C. elegans* strain

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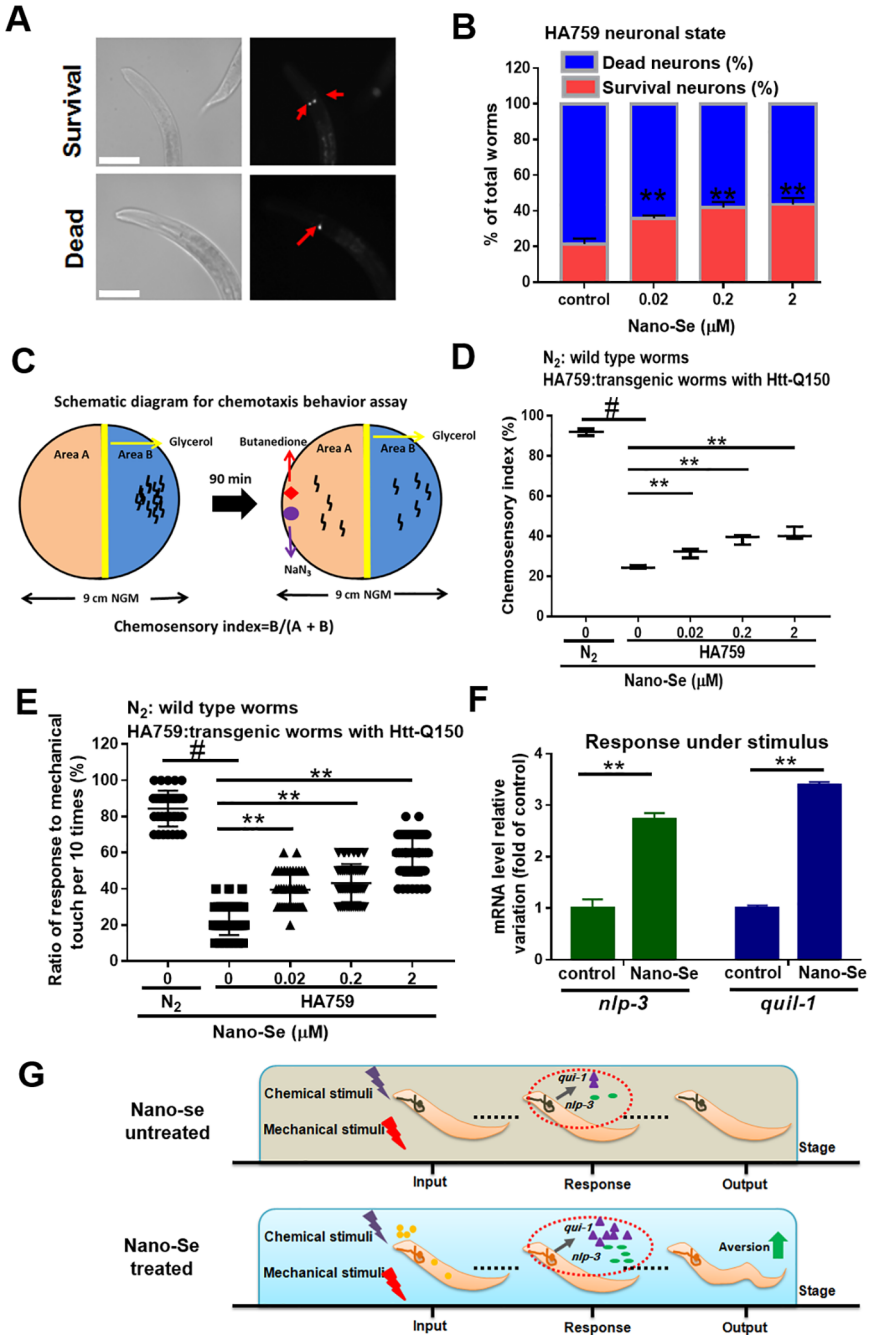

Figure 3. Effect of Nano-Se on ASH survival and sensory response function in transgenic *C. elegans* strain HA759. (A) Representative live fluorescence imaging of ASH neurons of HA759 nematodes. Death of ASH neurons was assessed by the loss of bilateral GFP fluorescence. (B) Survival rate of ASH neurons after Nano-Se treatment. (C) Schematic diagram for chemotaxis behavior assay system. The worms at the B region can be attracted to the A region by butanedione for 90 min. After the worms approached the A region, they will be paralyzed by NaN_3. The number of nematodes in areas A and B were counted and calculated for the chemosensory index as B/(A + B). (D–F) Effect of Nano-Se on (D) chemosensory behavior and (E) mechanical sensory behavior in *C. elegans*. Among all the worms, the ratio of worms that responded to the mechanical touch was counted upon the touch every 10 times. (F) Quantitative real-time PCR results of *nlp-3* and *qui-1* under chemo stimulus in HA759 after Nano-Se treatment. (G) Schematic diagram for the protective effect of Nano-Se on behavioral dysfunction in HA759 *C. elegans*. Data represent mean value ± standard errors. The double asterisks (**) indicate a very significant difference ($p < 0.01$) between untreated control and Nano-Se treated ones for HA759 worms. The pound key (#) represents a very significant difference ($p < 0.01$) between N2 wild-type and the untreated HA759 worms. The scale bar represents 100 μm.

HA759 was adopted to study the protection of Nano-Se from the damage to neurons. In HA759 *C. elegans*, huntingtin fragments Htt-Q150 (a polyQ150 tract derived from human Huntington) were highly expressed in ASH neuron, which leads to ASH neuronal death, but were lowly expressed in other neurons. We treated *C. elegans* with Nano-Se from the early L1 stage, in which stage among the whole life the nervous system is the most sensitive and vulnerable to detrimental effects.[29] Before or after Nano-Se treatment, the survival rate of ASH neurons was evaluated by the number of GFP (Figure

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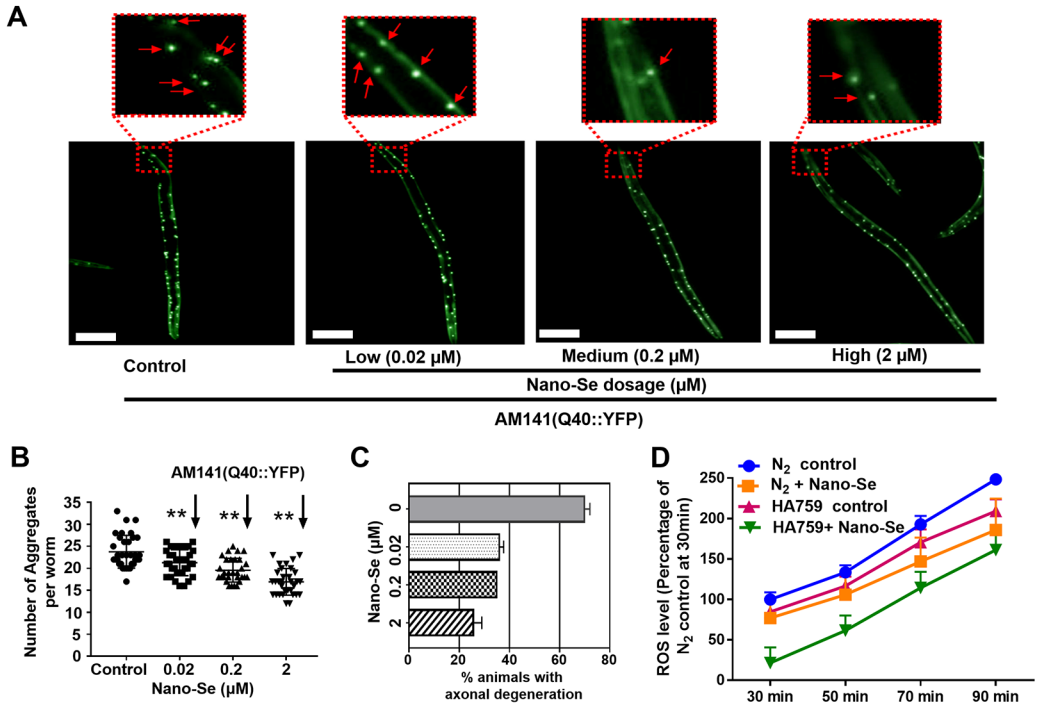

Figure 4. Decreased mutant huntingtin aggregates burden and oxidative stress in *C. elegans* strain AM141 by Nano-Se. (A) Representative fluorescence microscopic images of polyQ_40::YFP aggregates in Nano-Se treated and control groups. As a control group, untreated AM141 worms expressing polyQ_40::YFP display discontinuous and punctate fluorescent signals in the muscle cell of the body wall. (B, C) Quantitation of polyQ_40::YFP fluorescent aggregates in the groups after the exposure to three dosages of Nano-Se or not. The representativeness of the aggregates was described as the average value and standard errors. The fluorescent points along the worm body stands for the muscle-specific polyglutamine (Q40)::YFP aggregates that are labeled with green fluorescent protein (GFP). The number of fluorescent aggregates in the whole worm was calculated for the quantitation. Totally, 20 nematodes were randomly selected and scored for each tested group. (D) Quantitative ROS level in Nano-Se treated and control groups. Data show mean value ± standard errors. Double asterisks (**) mean the very siginicant difference between control and the Nano-Se treated samples. $^{**}p < 0.01$. Scale bar represents 100 μm.

3A). We found that Nano-Se reduced neuronal death significantly in a dose-dependent manner. Only 21% of ASH neurons survived in untreated nematodes, while 2 μM Nano-Se improved the neuronal survival rate to 44% after treatment with Nano-Se for 3 days (Figure 3B).

Nano-Se Preventing *C. elegans* from Behavioral Dysfunction. As a part of the nervous system, ASH sensory neuron is required for nociception, mechanosensation, osmosensation, and chemosensation, which plays an important role in avoidance responses to a variety of noxious stimuli and regulates its avoidance ability to hyperosmotic conditions.[30] Elimination of ASH sensory neuron pair leads to abolishment of the avoidance behavior in *C. elegans*.[31] Although the worms' sensation and motion behaviors are simple, it is feasible to use them to investigate integrated responses to different sensory stimuli. To verify protective roles of Nano-Se from behavioral dysfunction, the sensory and mechanical responses of ASH neurons were thus observed when HA759 nematodes were treated with Nano-Se at 0, 0.02, 0.2, and 2 μM for 3 days.

As shown in Figure 3D, with respect to N_2 wild-type, ~90% of *C. elegans*' ASH neurons were in healthy status, while the majority of transgenic HA759 nematodes lost the ability to sense noxious stimuli due to ASH neuronal death with a chemosensory index of ~0.25. After 2 μM Nano-Se therapy, the number of HA759 worms increased together with improved functions for ASH neurons in a dose dependence, for which the chemosensory index increased to ~0.4. Moreover, with respect to mechanosensory measurement, there were ~80% of N2 nematodes and ~20% of HA759 *C. elegans* normally responded to physical touch (Figure 3E). Interestingly, after treatment with 2 μM Nano-Se, the percentage of HA759 worms reached up to ~60% with normal response to mechanical disturbance (Figure 3E), indicating a significant contribution of Nano-Se to the alleviation in neuronal destruction. Once sensitized with external odor stimulus, ASH will express *qui-1*-encoded protein and release *nlp-3*-encoded neuropeptides to stimulate ASH-mediated aversive behavior.[33,34] To confirm the behaviors of HA759 worms, the expression of *nlp-3* and *qui-1* was upregulated after Nano-Se treatment compared with mock treatment using chemo stimulus (Figure 3F), indicating that Nano-Se treatment benefited the recovery of physiological functions of ASH neurons such as normal response to external stimulus.

Together, these results demonstrated that the protective effects of Nano-Se against neuronal dysfunction (Figure 3G). It was noteworthy that nanosized Se induces opposite effects compared to Se-containing compounds that decreased the movement and accelerated the paralysis of *C. elegans* in previous reports.[35] The reason might be that a higher concentration of other Se compounds such as $(PhSe)_2$ and Na_2SeO_3 was adopted that caused deleterious effects. In addition, the forms of Se may affect physiological functions of *C. elegans* distinctly.

Nano-Se Alleviating the Aggregation Degree of Huntingtin Proteins and the ROS Level. The aggregation of proteins is a characteristic of neuronal dysfunction and disease severity. In HD, mutations in the HTT gene lead to the

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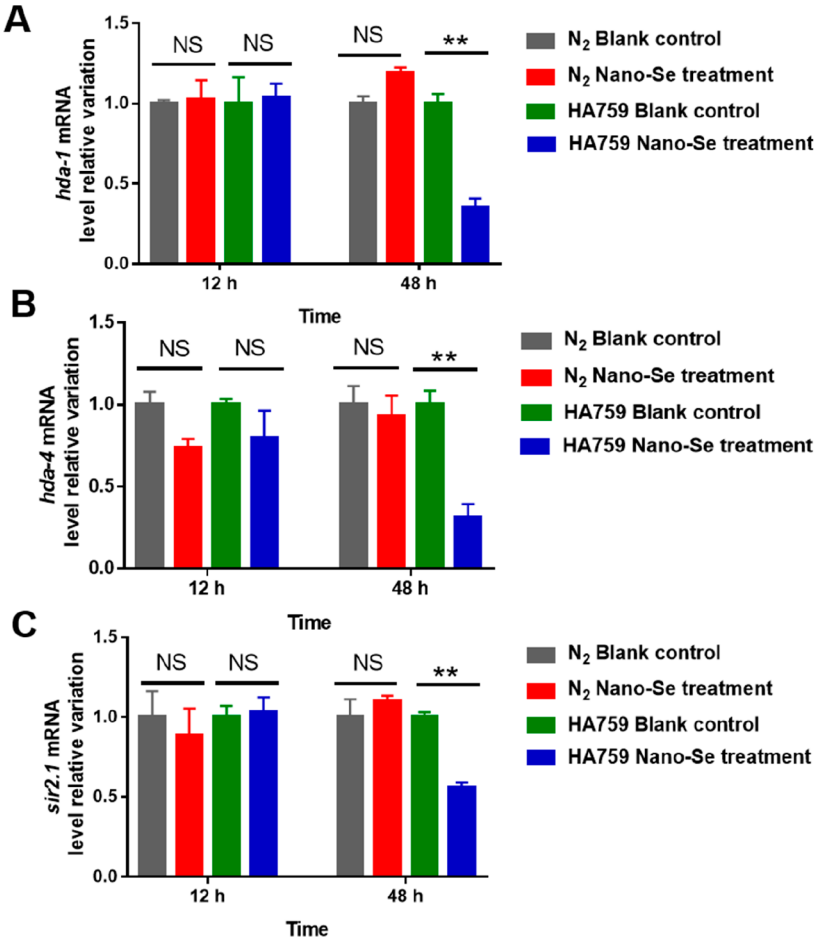

Figure 5. Effect of Nano-Se on mRNA expression of *hdc* family members. The mRNA levels of (A) *hda-1*, (B) *hda-4*, and (C) *sir2.1* in N_2 and HA759 groups after treatment with or without Nano-Se. Data represent mean value ± standard errors. $^{**}p < 0.01$. NS indicates no significance.

expansion of glutamine repeats and conformational rearrangements of proteins that form insoluble aggregates and disrupt normal functions of neurons. The mutations and subsequent aggregates are involved in the pathological processes including oxidative stress and ultimately cell death.[1]

In this study, transgenic strain AM141 *C. elegans* was adopted because the body wall muscle cells specifically express polyQ40::YFP fusion proteins that underwent the formation of discrete aggregates with fluorescent and the degeneration of the axonal processes when the worms reach adulthood. In the muscle of body wall, a highly punctate pattern with fluorescence is visualized and can be used to quantify polyQ aggregation (Figure 4A).[36] The level of aggregation can reflect the number of formed huntingtin fragments into discrete foci. After treatment with 0.02, 0.2, and 2 μM Nano-Se, the aggregation level was significantly reduced by 18%, 22%, and 30% compared with control, in a dose-dependent mode (Figure 4B,C). Consistently, more fluorescent proteins (YFP) appeared as soluble and the continuous fluorescent signals along the body wall after Nano-Se therapy compared to strong fluorescence signals for aggregation in untreated AM141 worms (30%) (Figure 4A–C). The decreasing number of discontinuous punctates along the body wall in the Nano-Se group indicated its role in decreasing axonal degeneration. In other words, Nano-Se treatment could effectively decrease HTT aggregation and reduce axonal degeneration in *C. elegans*. Previous reports showed that the surface of Nano-Se could easily form protein corona in biological systems, which may explain the inhibitory effects of Nano-Se on the HTT aggregation.[37]

Additionally, we verified the protective effects of Nano-Se supplement in *C. elegans* based on the antioxidative capacity of Nano-Se. Previous work reported that selenium serves as an important constituent of selenoproteins such as glutathione and thioredoxin systems that scavenge reactive oxygen species (ROS) such as $OH^{\bullet}$, $O_2^{\bullet -}$, and H_2O_2.[37] It was observed that after therapy by 2 μM Nano-Se, the ROS level of Huntington nematodes significantly decreased, demonstrating the capacity of Nano-Se for the clearance of ROS (Figure 4D). Antioxidant activity of Nano-Se *in vivo* might play an important role in attenuating huntingtin protein aggregation and neural degeneration. In contrast, Nano-Se caused negligible influence on the basal level of ROS in the wild-type N2 worms under normal culture condition. It was speculated that normal worms are less sensitive to beneficial nutrients such as Nano-Se, which aims at alleviating disorders, compared to worms suffering from internal or external adverse factors.

Nano-Se Alterating the Expression of Histone Deacetylase Family Members. In Huntington's disease, epigenetic factors emerge as important regulators for the transcription and metabolism of nutrition in response to cellular status and stress.[4] Downregulation of histone deacetylase (HDAC) members HDAC1 (analogue to HDA1), HDAC4 (analogue to HDA4), and SIRT1 (analogue

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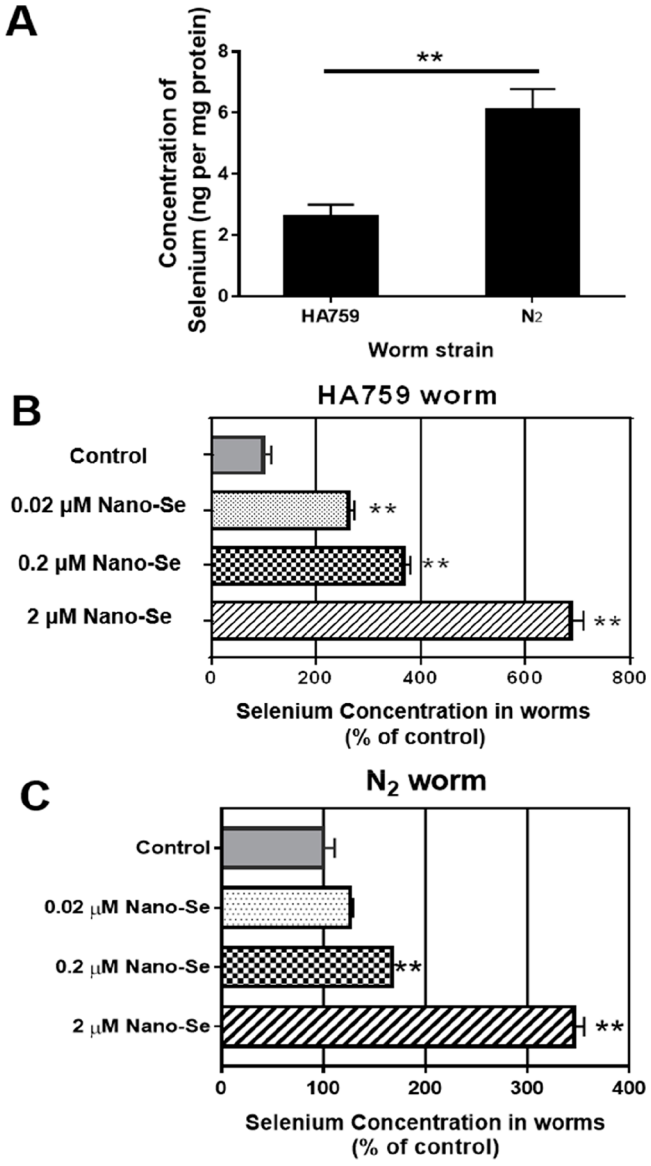

Figure 6. Intake and accumulation of selenium in the worms. (A) Content of selenium levels in the body of N_2 and HA759 *C. elegans*. The data were described as the mass of Se within 1 mg proteins of worm (ng Se/mg protein). (B) Accumulated selenium level in HA759 worm with selenite supplementation. (C) Accumulated selenium level in N_2 worm with selenite supplementation. Data represent mean value ± standard errors. $^{**}p < 0.01$.

to Sirt2.1) have been proved to suppress HD pathology in model organisms.[38−40] To demonstrate molecular mechanism about neural protection by Nano-Se, we compared mRNA level of several histone acetylation family members in wild-type N_2 and Huntington HA759 worms under Nano-Se treatment or not. It was found that the expression of *hda-1*, *hda-4*, and *sir2.1* genes significantly decreased in HA759 worms after the therapy with Nano-Se (Figure 5A−C). Comparably, mRNA levels of *hda-2*, *hda-3*, *hda-6*, and *hda-10* did not change in HA759 worms after treatment with Nano-Se (Figure S2). In contrast, the transcription of *hda* family members in wild-type worms showed no obvious change in both Nano-Se and control groups (Figure 5 and Figure S2). Taken together, at the epigenetic aspects, *hda* expression studies may help to understand the neuroprotection mechanism of Nano-Se from neural damage.

Nano-Se Therapy Promoting the Intake of Selenium by *C. elegans*. To know the contribution of accumulation of selenium to the therapy efficacy, we studied the intake of Nano-Se by *C. elegans*. It was obvious that selenium content in transgenic HA759 *C. elegans* was significantly lower than that in wild-type worms (Figure 6A). This was consistent with previous reports that the Se content in human HD brains at advanced stage decreases compared to normal brains.[41] After the therapy, the level of selenium in both N_2 and HA759 worms increased in dose dependence (Figure 6B,C). It is worth mentioning that the Se content of HA759 worms reached normal level as wild-type ones below 0.2 μM Nano-Se, while the Se content in N_2 worms changed much less after Nano-Se supplementation (Figure 6B,C). The difference in relative absorption capacity of selenium suggested the selectivity of nutrient requirements for selenium in HD worms. The reason might be that intestinal structures and function change in HD model that influence the intake and metabolism of selenium.[41,42] Finally, upon exposure to Nano-Se, increasing uptake and accumulation of selenium in HD model of *C. elegans* largely improve the level of selenium *in vivo*, which can explain how Nano-Se eliminates ROS and plays protective roles in recovering physiological functions of neurons in HD disease. However, further study will be addressed in the future to reveal how Nano-Se works and behaves *in vivo* to regulate the therapy of HD at molecular and cellular aspects.

CONCLUSIONS

In this paper, we found that Nano-Se below 2 μM can reduce neuronal death, relieve behavioral dysfunction, and offer protection to *C. elegans* under stress. A cause−effect relationship lies between oxidative stress, epidemic factors, and HTT proteins in the pathogenesis of neurodegenerative disorders. Not only overexpression of mutant HTT induces abnormal level of oxidative stress and epidemic change, which damages cellular components and triggers cell death, but also oxidative stress or high levels of HDAC can contribute to protein aggregates and impaired neuronal function.[42,43]. After treatment with Nano-Se, both the ROS level and HTT aggregation *in vivo* for transgenic Huntington *C. elegans* decreased, and downregulation of *hda* mRNA was also observed at epidemic levels. Therefore, we speculated that Nano-Se plays antioxidant roles in regulating the expression of *hda* family and is capable of reducing the degree of polyQ aggregation (Scheme 1). To conclude, our findings indicate that Nano-Se may be promising and attractive for the therapy of human HD through diets. Treatment of HD disease will benefit from the deep understanding of how Nano-Se alleviates HD disease and the relationship between physicochemical properties and the therapeutic efficacy. Moreover, the rational design of Nano-Se may also improve the dosage tolerability for HD therapy in comparison with other selenium species in the future.

ASSOCIATED CONTENT

Supporting Information

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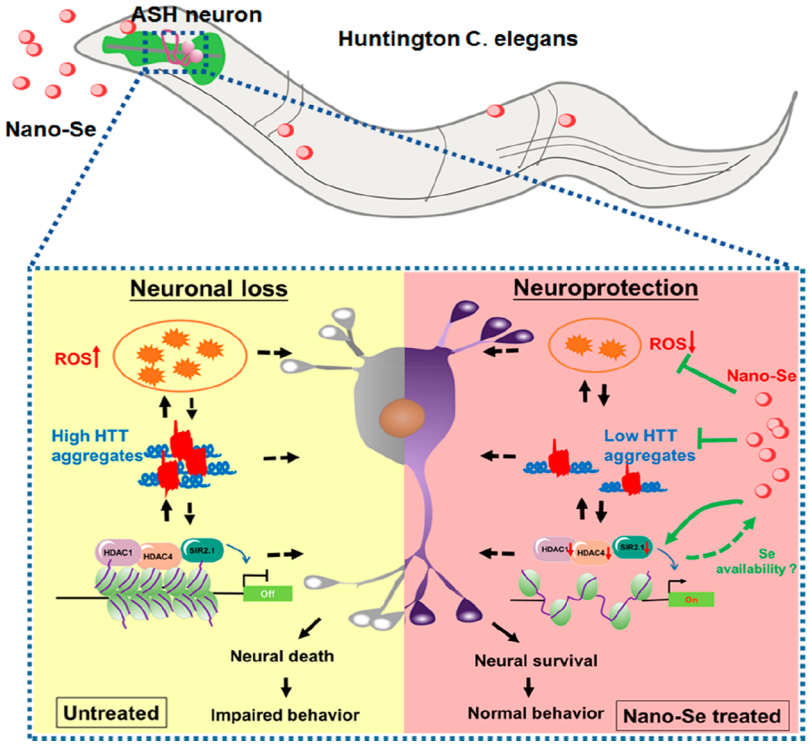
Scheme 1. Schematic Diagram for Neuroprotective Function of Nano-Se in Neurodegenerative Huntington's Disease Model of *C. elegans*



Surviving curve of *C. elegans* under MeHg and the effect of Nano-Se on gene expression of several *hda* family members (PDF)

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Notes

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