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Quantum dots exposure alters both development and function of D-type GABAergic motor neurons in nematode *Caenorhabditis elegans*†

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We examined the *in vivo* quantum dots (QDs) neurotoxicity and the underlying mechanism using *Caenorhabditis elegans* D-type GABAergic motor neurons as the assay system. Prolonged exposure to low concentrations of CdTe QDs caused damage on both the development and function of D-type motor neurons, and resulted in translocation of CdTe QDs into D-type motor neurons. In addition to oxidative stress, cell identity was also involved in the induction of the toxicity of QDs on D-type motor neurons. ZnS surface coating blocked CdTe QDs translocation and maintained cell identity, thereby suppressing CdTe QDs neurotoxicity. For the underlying mechanism, we hypothesized that both translocation into the targeted neurons and alterations in the development and function of those targeted neurons contribute to the induction of CdTe QDs neurotoxicity. Considering the conserved property of GABAergic neurons during evolution, our data will shed light on our understanding of the potential risks of QDs to the nervous systems of animals.

Introduction

Quantum dots (QDs), a class of inorganic fluorophore and among the most promising items in the nanomedicine toolbox, are tiny light-emitting particles. QDs can be potentially applied in nanodiagnostics, imaging, proteomic and genomic assays, drug delivery, targeted therapy and pathology.[1–3] With the increased use of QDs in biological and medical applications, both *in vitro* and *in vivo* toxicities caused by QDs have received great attention.[4–6] *In vitro* and *in vivo* studies have elucidated the cellular transport kinetics, cellular mechanisms of some toxicities, and biodistribution of QDs.[5,6] QDs could be accumulated in various organs, including spleen, liver and kidney, in mice.[7,8] QDs have been shown to cause mitochondrial alterations, pulmonary vascular thrombosis, lung inflammation, hepatotoxicity, genotoxicity, and ecotoxicity.[5–7,9–11] Moreover, it has been found that QDs induced the elevation of cytoplasmic calcium levels and impaired synaptic transmission and plasticity in rat hippocampal neurons.[12,13]

Caenorhabditis elegans, a model organism with a simple and well-defined anatomy, has been employed as a benchmark system for toxicological study because of its short life cycle, ease of handling, and high sensitivity to various stresses.[14–17] *C. elegans* can be used for toxicological studies, including neurotoxicological study from whole-animal level down to single-cell level.[18–23] To date, *C. elegans* has been successfully used in environmental and toxicological studies of different engineered nanomaterials (ENMs) such as metal nanoparticles (NPs), carbon-based nanomaterials, and nanocrystals.[24–32] *C. elegans* has been further shown to be helpful for elucidating the toxicological mechanisms of neurotoxicity from ENMs.[33,34]

Recently, several studies have reported the toxicity of QDs on the growth, reproduction, and lifespan of *C. elegans*.[35–37] Qu *et al.* also examined the metabolism and degradation of QDs in alimentary system of nematodes.[35] However, we still know little about the *in vivo* neurotoxicity of QDs and its underlying mechanism. In *C. elegans*, γ-aminobutyric acid (GABA), an amino acid neurotransmitter, acts primarily at neuromuscular synapses.[38] Among the 26 GABAergic neurons, the ventral cord D-type neurons, including 6 DD and 13 VD motor neurons, inhibit contraction of the ventral and dorsal body wall muscles during locomotion.[38] When a nematode lacking functional D-type motor neurons is touched, it displays a shrinking behavior.[38] In the present study, we report an *in vivo* neurotoxicological study of QDs in the model organism of *C. elegans*. We investigated the underlying mechanism of QDs-induced shrinking behavior and disrupted development and

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function of D-type motor neurons in nematodes. Considering the conserved property of GABAergic neurons during the evolution,[38] our results will provide important insight into understanding the potential risks and possible mechanisms of QDs toxicity in the development and function of nervous systems in other biological assay systems.

Materials and methods

Characterization of QDs

CdTe QDs and CdTe@ZnS QDs (CdTe QDs with ZnS coating) were from Jiayuan Quantum Dots Co., China. Both CdTe QDs and CdTe@ZnS QDs were stabilized by negatively-charged 3-mercaptopropionic acid (MPA). QDs were characterized by transmission electron microscopy (TEM) (JEM-200CX, JEOL, Japan). Images were collected using the field emission JEM-200CX TEM, equipped with a CCD camera. UV-vis absorption spectra and fluorescence spectra were carried out with a TECAN microplate reader (Tecan, Durham, USA). Zeta potentials of QDs were analyzed by Nano Zetasizer using a dynamic light scattering (DLS) technique. The prepared QDs suspension concentrations were 0.001, 0.01, 0.1, 1, 10, and 100 μg L^{-1} in K-medium. All the other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Strain preparation

Nematodes used in the present study were wild-type N2 and transgenic strains of *oxIs12*[*Is*(P*unc-47::GFP*)], *dvIs15*[*Is*(P*mtl-2::GFP*)], *Ex*(P*unc-25-unc-30*), *Ex*(P*unc-25-sod-2*), and *Ex*(P*unc-25-sod-3*). They were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 as described.[14] The age synchronous populations of L1-larvae were obtained as described previously.[39] Previous studies have demonstrated that prolonged exposure assay system from L1-larvae to young adult stage can be used to assess the toxicity of environmentally relevant concentrations of ENMs.[30,31] In this study, prolonged exposures from L1-larvae to young adult stage were performed in 12-well sterile tissue culture plates at 20 °C in the presence of food.

Lethality and paralysis assays

To assess the lethality, after exposure, wells were observed under a dissecting microscopy. Nematodes were judged to be dead if they did not respond to a stimulus using a small metal wire. Nematodes were judged to be paralyzed if they did not move after being touched with a small metal wire, although they still had the normal pharynx pumping behavior. Fifty nematodes were examined for each treatment. Five replicates were performed.

Shrinking behavior assay

When a nematode is tapped on the nose, it will normally move backwards by propagating a sinusoidal wave from the tail to the head. When the function of D-type motor neurons is disrupted, nematodes will show shrinking behavior.[38] Shrinking behavior is defined as the phenotype that nematode pulls in its head and shorten its body due to hyper-contraction of the body wall muscles on both the sides of the body.[38] Thirty nematodes were examined for each treatment. Five replicates were performed.

Reactive oxygen species (ROS) production

As described previously,[40,41] the examined nematodes were transferred to 1 mL of CM-H2DCFDA (1 μmol L^{-1}) prepared with M9 buffer in 12-well sterile tissue culture plates to pre-incubate for 3 h at 20 °C. Nematodes were then mounted on 2% agar pads for the observation with a laser scanning confocal microscope (Leica, TCS SP2, Bensheim, Germany) at 488 nm of excitation wavelength and 510 nm of emission filter. The semi-quantified ROS was expressed as the relative fluorescent units (RFU). Twenty nematodes were used for each treatment. Five replicates were performed.

Reverse-transcription and quantitative real-time polymerase chain reaction (PCR)

Total RNA of nematodes was extracted using RNeasy Mini Kit (Qiagen), and reverse-transcribed using a cDNA synthesis kit (Bio-Rad Laboratories). The optimized annealing temperature for the run of quantitative reverse transcription PCR (RT-PCR) was 58 °C. The final results were expressed as the relative expression ratio between targeted gene and *tba-1* gene encoding a tubulin protein. The designed primers for the targeted genes and reference *tba-1* gene are shown in Table S1.† Three replicates were performed.

Analysis of the axonal degeneration and neuronal loss of D-type GABAergic neurons

We used the transgenic strain of *oxIs12* to visualize the D-type motor neurons in nematodes. The gap number of ventral or dorsal cord was quantified to reflect the axonal degeneration. The number of cell bodies in D-type GABAergic nervous system was quantified to assess the possible neuronal loss. The fluorescent images were photographed on the same day to avoid the possible effects of light source variance. Thirty nematodes were examined for each treatment. Three replicates were performed.

DNA construct and germline transformation

The *unc-25* promoter was used to drive genes to express in D-type motor neurons. The *unc-25* promoter (1.9 kb) was amplified by PCR from *C. elegans* genomic DNA, and then inserted into the pPD95_77 vector in the sense orientation. The *unc-30* cDNA was amplified by PCR, verified by sequencing, and inserted into the vector carrying *unc-25* promoter sequence. Germline transformation was performed by co-injecting the testing DNA at a concentration of 10–40 μg mL^{-1} and the marker DNA of P*unc-119-unc-119* at a concentration of 60 μg mL^{-1} into the gonad of nematodes, as described previously.[42]

Determination of the internalized Cd by nematodes

To determine the Cd uptake, the nematodes were exposed to 1 μg L^{-1} of CdTe QDs from L1 larvae to young adult stage. The contents of Cd in nematodes were analyzed using an inductively coupled plasma-mass spectrometry (ICP-MS, Elan6100, PerkinElmer, USA). After exposure, approximately five thousand of the nematodes were transferred to clean NGM-plates to remove the bacteria. The nematodes were washed with ice-cold ultrapure water four times and transferred into pre-weighed glass tubes to digest in a block heater at 90 °C for 4 h after the addition of 1 mL of 70% HNO_3. After digestion, the samples were filtered and transferred to polypropylene tubes and diluted with ultrapure water to achieve a final acid concentration of 1% by volume by adjusting with nitric acid.

Statistical analysis

Data in this study were presented as means ± standard error of the mean (S.E.M.). We used the SPSS 12.0 software (SPSS Inc., Chicago, USA) to perform the statistical analysis. Differences between groups were determined using the analysis of variance (ANOVA). Probability levels of 0.05 and 0.01 would be considered to be statistically significant.

Results

Physicochemical properties of QDs

CdTe@ZnS core–shell QDs and CdTe QDs are two types of the most commonly used NPs and were stabilized by negatively charged MPA (Fig. 1A). The QDs used had similar size (CdTe QDs, 3.7 ± 0.8 nm; CdTe@ZnS QDs, 3.9 ± 0.9 nm) (Fig. 1B). UV-vis absorption spectra of CdTe QDs and CdTe@ZnS QDs are shown in Fig. 1C, and fluorescence spectra of CdTe QDs and CdTe@ZnS QDs are shown in Fig. 1D. Zeta potentials of CdTe QDs and CdTe@ZnS QDs in K-medium were −12.9 and −24.5 mV, respectively (Fig. 1E). The suspension solution of CdTe QDs or CdTe@ZnS QDs was stable at least for one week without altered zeta potential or evident aggregation or deposition.

CdTe QDs exposure induced the shrinking behavior in nematodes

In *C. elegans*, prolonged exposure to CdTe QDs at concentrations of 0.001–10 μg L^{-1} did not induce lethality, and prolonged exposure to CdTe QDs at concentrations of 0.001–1 μg L^{-1} did not induce paralysis (Fig. 1F). In contrast, 10–100 μg L^{-1} of CdTe QDs induced paralysis, and 100 μg L^{-1} of CdTe QDs caused lethality (Fig. 1F). We then selected the concentrations of 0.001–1 μg L^{-1} for analyzing the possible adverse effects of CdTe QDs on the development and function of D-type motor neurons.

In *C. elegans*, the appearance of shrinking behavior was due to the dysfunction of D-type motor neurons.[38] We observed that, although prolonged exposure to 0.001–0.01 μg L^{-1} of CdTe QDs did not induce the shrinking behavior, prolonged exposure to 0.1–1 μg L^{-1} of CdTe QDs resulted in the formation of shrinking behavior in nematodes (Fig. 1G and H), indicating the dysfunction of D-type motor neurons in nematodes exposed to 0.1–1 μg L^{-1} of CdTe QDs.

Effects of CdTe QDs exposure on the development of D-type motor neurons

With the aid of transgenic strain of *oxIs12*[*Is*(P*unc-47::GFP*)] labeling GABAergic D-type motor neurons, we investigated the possible adverse effects of prolonged exposure to CdTe QDs on the development of D-type motor neurons. We observed that relatively high concentrations of CdTe QDs noticeably affected the development of D-type motor neurons. Although prolonged exposure to 0.001–0.01 μg L^{-1} of CdTe QDs did not influence the fluorescent intensity of D-type motor neurons and induce the neuronal loss, prolonged exposure to 0.1–1 μg L^{-1} of CdTe QDs significantly decreased the fluorescent intensity of cell bodies for D-type motor neurons and induced the neuronal loss and the formation of gaps on both ventral and dorsal cords (Fig. 2A–D). Thus, along with the dysfunction of D-type motor neurons, the severe deficits in the development of D-type motor neurons were found in CdTe QDs exposed nematodes.

CdTe QDs exposure altered expression patterns of genes required for the synthesis, transport and development of D-type motor neurons

In *C. elegans*, *unc-30* gene encodes a homeodomain transcription factor that is required for the GABA neuron identity.[43] The *unc-25* gene encodes a glutamic acid decarboxylase, the biosynthesis enzyme for GABA.[44] The *unc-47* gene encodes a GABA transporter that pumps GABA into the synaptic vesicles.[45] Prolonged exposure to 1 μg L^{-1} of CdTe QDs significantly decreased the expression level of *unc-30* gene compared with the control (Fig. 2E), suggesting the damage on cell identity of D-type motor neurons in CdTe QDs exposed nematodes. Interestingly, we further found that prolonged exposure to 1 μg L^{-1} of CdTe QDs significantly decreased the expression levels of *unc-25* and *unc-47* genes compared with controls (Fig. 2E). These data indicate that CdTe QDs exposure may cause the damage on both cell fate and synthesis and transport of GABA neurotransmitter.

Effects of overexpressing UNC-30 in D-type motor neurons on the neurotoxicity from CdTe QDs

To further examine the role of development of D-type motor neurons in toxicity formation from CdTe QDs, we constructed the transgenic strain of *Ex*(P*unc-25-unc-30*), in which the *unc-30* gene was overexpressed in D-type motor neurons to strengthen the identity of neurons. Overexpression of *unc-30* in D-type motor neurons did not induce the shrinking behavior (Fig. 2F). Moreover, after prolonged exposure to 1 μg L^{-1} of CdTe QDs, we did not observe the formation of shrinking behavior in nematodes overexpressing the *unc-30* gene in D-type motor neurons (Fig. 2F and G). Moreover, we did not observe deficits in the development of D-type motor neurons in *Ex*(P*unc-25-unc-30*) nematodes exposed to 1 μg L^{-1} of CdTe

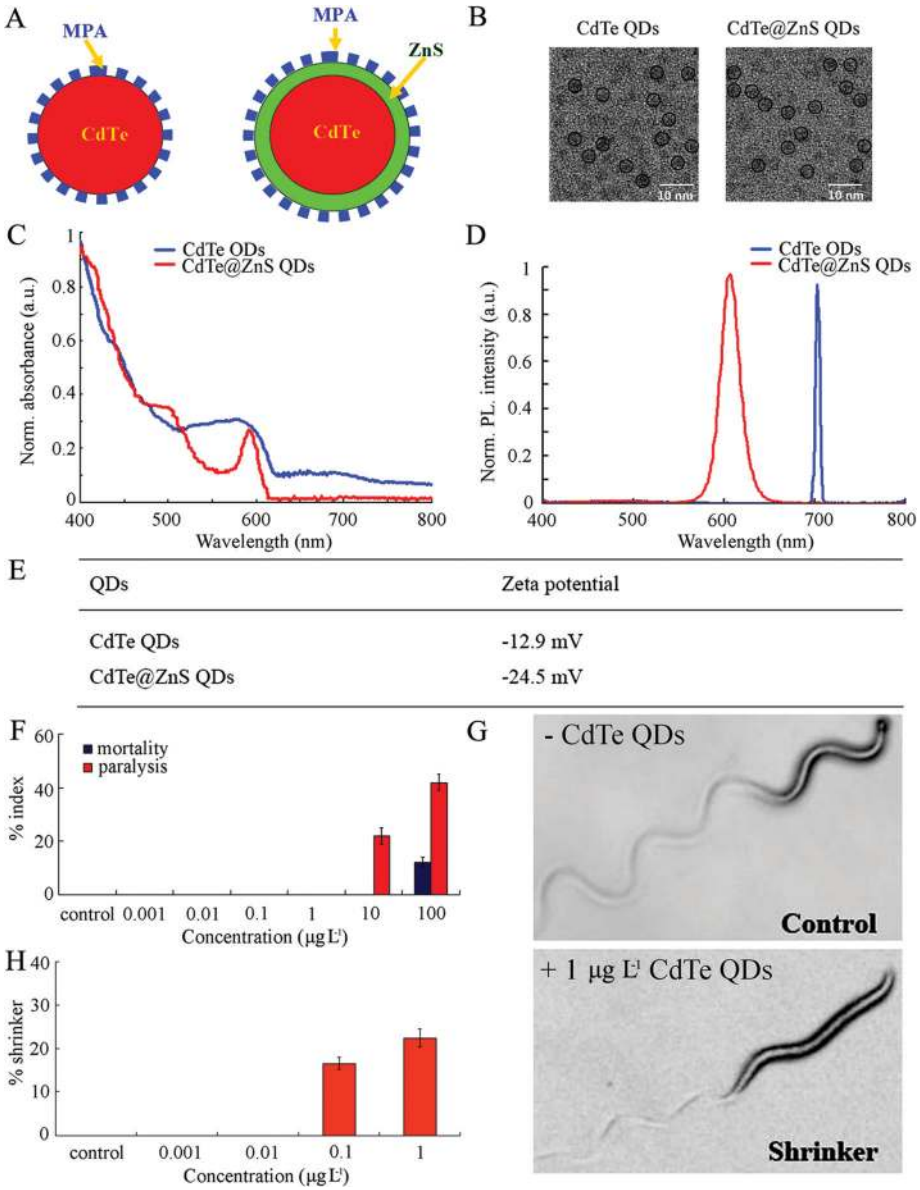

Fig. 1 CdTe QDs exposure induced the shrinking behavior in *C. elegans*. (A) Schematic drawing of the structure of two types of water soluble QDs. MPA, 3-mercaptopropionic acid. (B) TEM images of CdTe QDs and CdTe@ZnS QDs. (C) UV-vis absorption spectra of CdTe QDs and CdTe@ZnS QDs. (D) Fluorescence spectra of CdTe QDs and CdTe@ZnS QDs. (E) Zeta potentials of CdTe QDs and CdTe@ZnS QDs. (F) Mortality and paralysis induced by CdTe QDs. (G) Shrinking behavior induced by CdTe QDs. (H) Pictures showing the normal and shrinking behaviors of nematodes. Control, without CdTe QDs exposure. CdTe QDs was exposed from L1-larvae to young adult stage. Bars represent means ± SEM.

QDs (Fig. S1†). Thus, strengthening the identity of D-type motor neurons may be useful for counteracting the toxicity of CdTe QDs in nematodes.

Distribution and translocation of CdTe QDs in D-type motor neurons

To investigate the underlying mechanism of toxicity of CdTe QDs on the development and function of D-type motor neurons, we next examined the distribution of CdTe QDs. We observed that, after prolonged exposure to 1 μg L^{-1} of CdTe QDs, the red fluorescence from CdTe QDs could be co-localized with the green fluorescent puncta labeling of the D-type motor neurons (Fig. 3), demonstrating that CdTe QDs could be translocated into the D-type motor neurons.

ROS production could be induced together with the translocation of CdTe QDs

Previous studies have demonstrated that oxidative stress may play a key role in inducing QDs toxicity.[4–6] In *C. elegans*, prolonged exposure to 0.001–0.01 μg L^{-1} of CdTe QDs did not induce ROS production; however, prolonged exposure to 0.1–1 μg L^{-1} of CdTe QDs caused the significant induction of

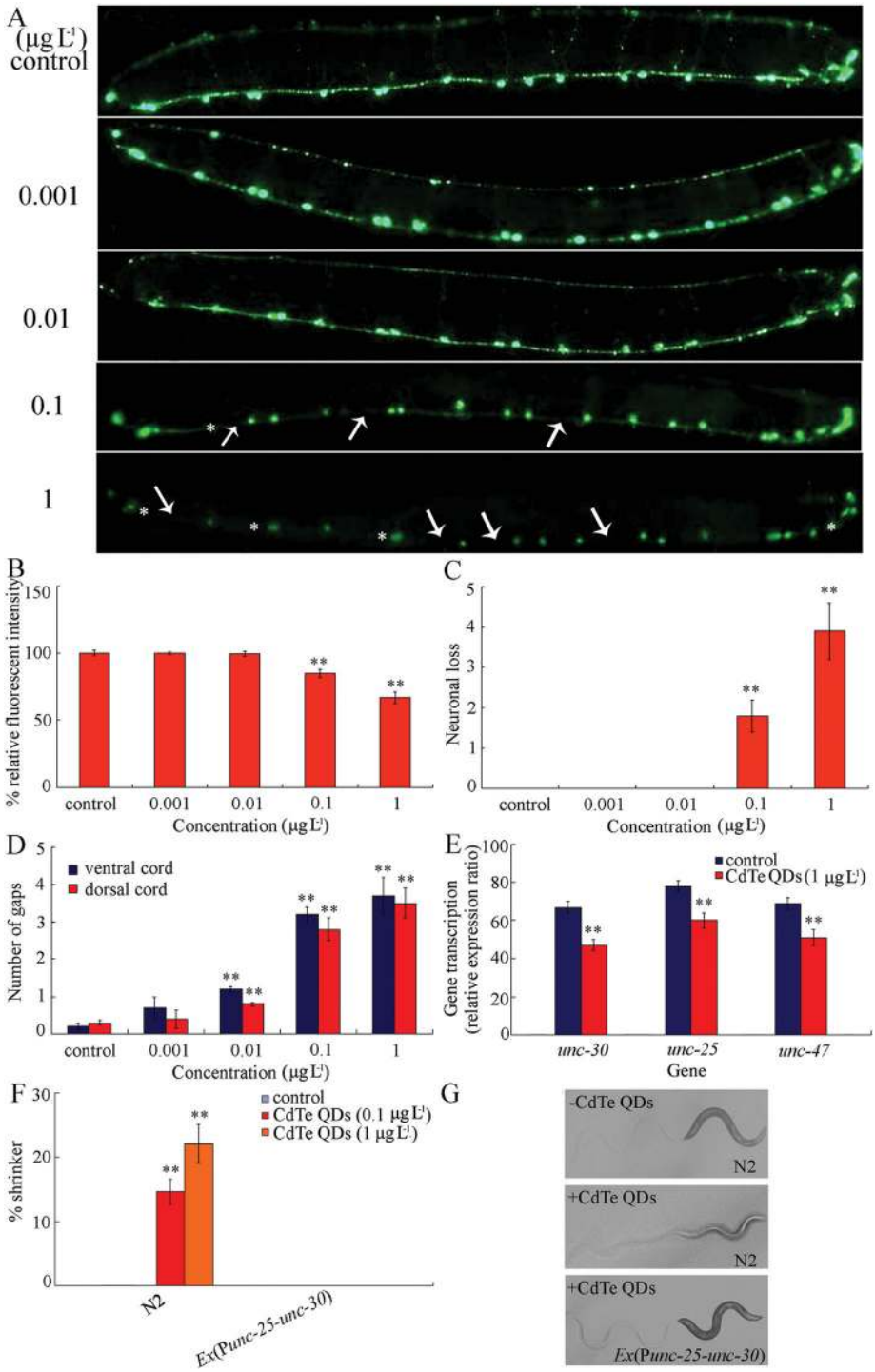

Fig. 2 Effects of CdTe QDs exposure on the development of D-type motor neurons. (A) Images showing the effects of CdTe QDs exposure on the development of D-type motor neurons. Asterisks indicate the neuronal loss, and arrowheads indicate the gap formation on ventral cord. (B) Effects of CdTe QDs exposure on the fluorescent intensity of cell bodies for ventral D-type motor neurons. (C) Effects of CdTe QDs exposure on the neuronal loss of D-type motor neurons. (D) Effects of CdTe QDs exposure on the gap formation in both ventral and dorsal cords of D-type motor neurons. (E) Effects of CdTe QDs exposure on the expression pattern of *unc-30*, *unc-25*, and *unc-47* genes. The qRT-PCR results were expressed as the relative expression ratio between targeted gene and reference *tba*-1 gene. (F) Formation of shrinking behavior in CdTe QDs exposed wild-type or *Ex*(P*unc-25-unc-30*) transgenic strain. (G) Pictures showing the effects of overexpressing *unc-30* gene in D-type motor neurons on the formation of shrinking behavior in CdTe QDs exposed nematodes. Control, without CdTe QDs exposure. CdTe QDs was exposed from L1-larvae to young adult stage. Bars represent means ± SEM. $^{**}P < 0.01$.

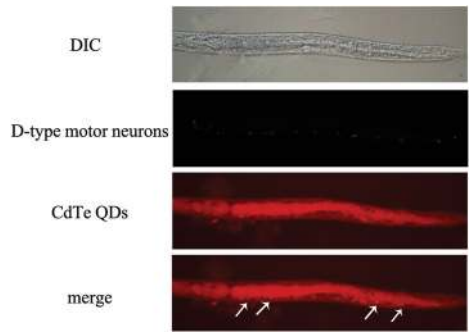

Fig. 3 Distribution and translocation of CdTe QDs in D-type motor neurons of nematodes. CdTe QDs was exposed from L1-larvae to young adult stage. Arrowheads indicate the merge positions.

ROS production compared with control (Fig. 4A and B). More interestingly, we observed that the induced ROS signals could be largely co-localized with the translocated CdTe QDs into the body (Fig. 4C). This indicated the possible involvement of oxidative stress in inducing neurotoxicity on the development and function of D-type motor neurons.

Overexpression of SOD-2 or SOD-3 prevented the formation of toxicity on D-type motor neurons in CdTe QDs exposed nematodes

In *C. elegans*, *sod*-2 and *sod*-3 genes encode the Mn-SODs, which function against the toxicity formation from toxicants.[30,46] Overexpression of *sod*-2 or *sod*-3 gene in D-type motor neurons did not induce the shrinking behavior (Fig. 4E). After prolonged exposure to 1 μg L^{-1} of CdTe QDs, we did not observe the shrinking behavior in nematodes overexpressing the *sod*-2 or *sod*-3 gene in D-type motor neurons (Fig. 4E and F). Moreover, we did not detect the deficits in the development of D-type motor neurons in *Ex*(P*unc-25-sod-2*) or *Ex*(P*unc-25-sod-3*) strains exposed to 1 μg L^{-1} of CdTe QDs (Fig. S1†). Therefore, induction of oxidative stress in D-type motor neurons may be important for the neurotoxicity formation of CdTe QDs on the development and function of D-type motor neurons in nematodes.

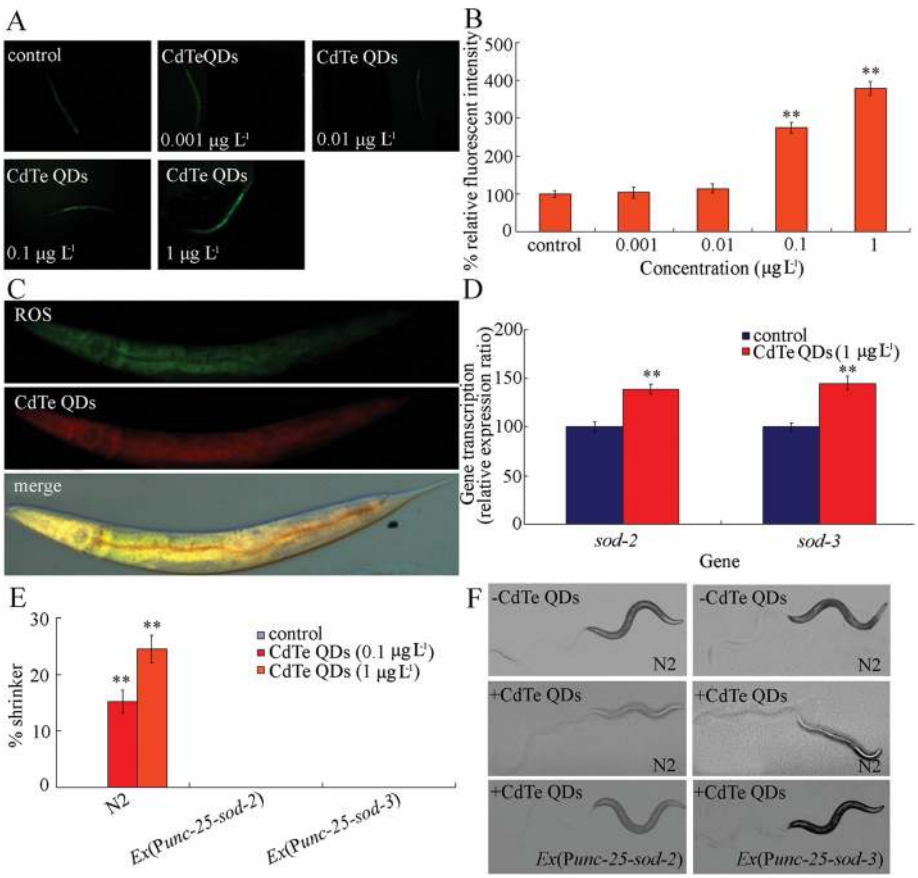

Fig. 4 Contribution of oxidative stress to the toxicity formation of CdTe QDs. (A) Pictures showing the ROS production in CdTe QDs exposed nematodes. (B) Comparison of ROS production between control and CdTeQDs exposed nematodes. (C) Colocalization of ROS production with CdTe QDs. (D) Expression patterns of *sod*-2 and *sod*-3 genes in CdTe QDs exposed nematodes. The qRT-PCR results were expressed as the relative expression ratio between targeted gene and reference *tba*-1 gene. (E,F) Effects of overexpressing *sod*-2 and *sod*-3 genes in D-type motor neurons on the shrinking behavior formation in CdTe QDs exposed nematodes. Control, without CdTe QDs exposure. CdTe QDs was exposed from L1-larvae to young adult stage. Bars represent means ± SEM. $^{**}P < 0.01$.

ZnS coating reduced toxicity of CdTe QDs on the development and function of D-type motor neurons

Previous studies have demonstrated that specific surface modifications such as ZnS coating can reduce the toxicity of QDs.[45,46] We further tested whether the ZnS coating could reduce the toxicity of CdTe QDs on D-type motor neurons. After prolonged exposure, none of the examined concentrations of CdTe@ZnS QDs induced the shrinking behavior (Fig. 5A). Moreover, prolonged exposure to any of the examined concentrations of CdTe@ZnS QDs did not cause evident morphological changes (Fig. 5B), alter the relative fluorescent intensities of cell bodies (Fig. 5C), induce the neuronal loss (Fig. 5D), and result in the formation of severe gaps on both ventral and dorsal cords of D-type motor neurons (Fig. 5E). In addition, prolonged exposure to CdTe@ZnS QDs did not influence the expression patterns of *unc-30*, *unc-25*, and *unc-47* genes compared with control (Fig. 5F). Thus, ZnS coating can reduce the toxic effects of CdTe QDs on both the development and function of D-type motor neurons in nematodes.

Cellular mechanism of ZnS to reduce the neurotoxicity of CdTe QDs on D-type motor neurons

To determine the possible mechanism by which ZnS coating reduces this CdTe QDs toxicity, we investigated the distribution

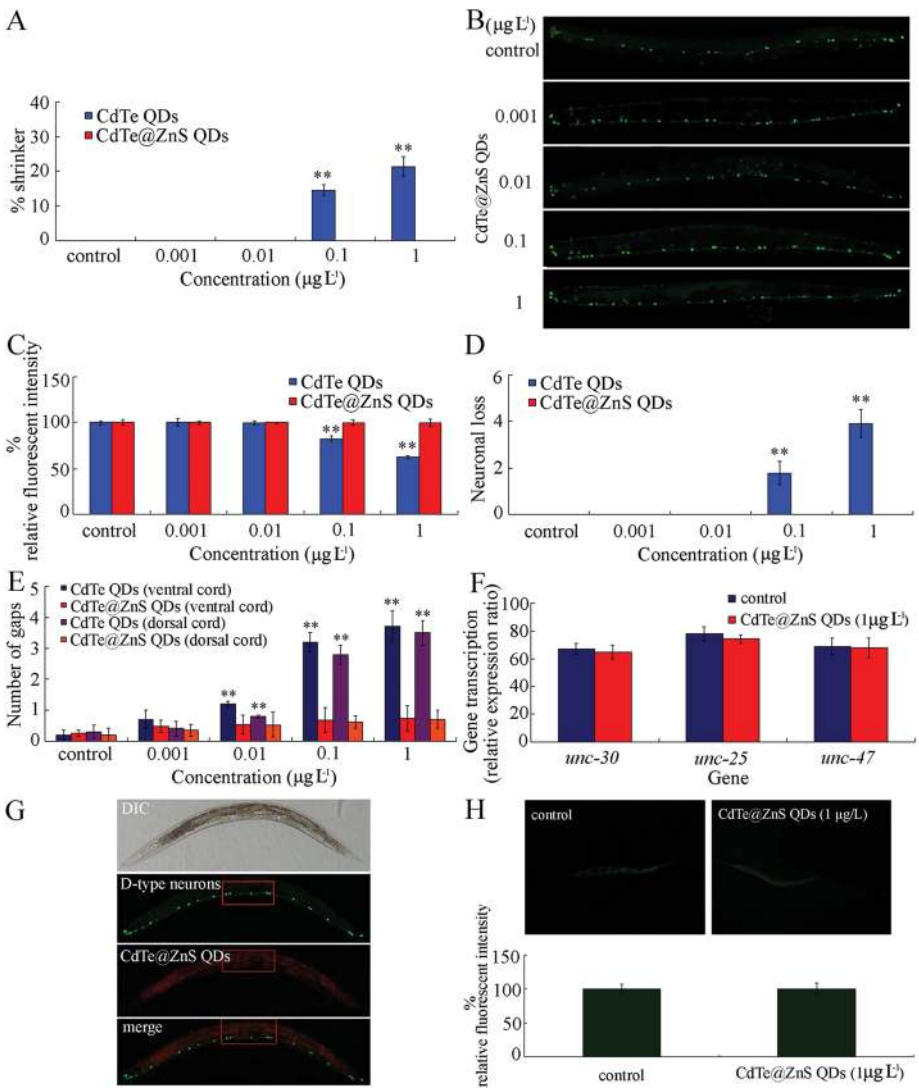

Fig. 5 ZnS coating reduced toxicity of CdTe QDs on the development and function of D-type motor neurons. (A) Comparison of effects on the shrinking behavior formation between CdTe QDs and CdTe@ZnS QDs. (B) Images showing effects of CdTe@ZnS QDs on the development of D-type motor neurons. (C) Comparison of effects on the fluorescent intensity of cell bodies in D-type motor neurons between CdTe QDs and CdTe@ZnS QDs. (D) Comparison of effects on the neuronal loss of D-type motor neurons between CdTe QDs and CdTe@ZnS QDs. (E) Comparison of effects on the formation of gaps on ventral and dorsal cords between CdTe QDs and CdTe@ZnS QDs. (F) Effects of CdTe@ZnS QDs on the expression levels of *unc-30*, *unc-25*, and *unc-47* genes. The qRT-PCR results were expressed as the relative expression ratio between targeted gene and reference *tba-1* gene. (G) CdTe@ZnS QDs (1 μg L^{-1}) could not be colocalized with D-type motor neurons. (H) Effects of CdTe@ZnS QDs on the ROS production. Control, without QDs exposure. CdTe QDs was exposed from L1-larvae to young adult stage. Bars represent means ± SEM. $^{**}P < 0.01$.

and translocation of CdTe@ZnS QDs in nematodes. In *C. elegans*, CdTe@ZnS QDs was mainly distributed in the intestine (Fig. 5G). In addition, the red fluorescent signals of CdTe@ZnS QDs could not be colocalized with the green fluorescent signals of D-type motor neurons, suggesting that CdTe@ZnS QDs was not translocated into the D-type motor neurons (Fig. 5G). Furthermore, we observed that prolonged exposure to 1 μg L^{-1} of CdTe@ZnS QDs did not induce noticeable ROS production compared with the control (Fig. 5H). These data indicate that the reduction of CdTe QDs neurotoxicity on D-type motor neurons by ZnS coating may be due to both the blockage of translocation into and the activation of ROS production in D-type motor neurons in nematodes.

Toxicity of the examined concentrations of CdTe QDs to induce the neurotoxicity on D-type motor neurons might be not due to the cadmium ion release

One of the possible reasons for QDs-induced toxicity might be the release of Cd^{2+}.[9,47] In *C. elegans*, the transgenic strain of *dvIs15*[*Is*(P*mtl-2::GFP*)] can be used to specifically detect the possible existence of toxicity from Cd^{2+}.[48] Under normal conditions, P*mtl-2::GFP* has only a very weak expression; however, Cd^{2+} at concentrations causing toxicity on nematodes could induce a sharp increase in expression of P*mtl-2::GFP*.[48] We found that 1 μg L^{-1} of CdTe QDs could not induce the evident expression of P*mtl-2::GFP* in nematodes (Fig. S2†), suggesting that perhaps only a very small amount of Cd^{2+} could be released from 1 μg L^{-1} of CdTe QDs and the released concentration of Cd^{2+} could not induce adverse effects on nematodes. Based on the ICP-MS assay, we further found out that the body Cd burden/worm was approximately 3.4×10^{-4} ng per worm only. These data indicate that the toxic effects observed on the development and function of D-type motor neurons from the examined concentrations of CdTe QDs may not be due to the possible released Cd^{2+}.

Discussion

In the present study, we observed the induction of shrinking behavior by prolonged exposure to 0.1–1 μg L^{-1} of CdTe QDs from L1-larvae to young adult stage(Fig. 1G and H). Moreover, we found that prolonged exposure to 0.1–1 μg L^{-1} of CdTe QDs resulted in severe deficits in the development of D-type motor neurons that are involved in the control of shrinking behavior (Fig. 2A–D). Prolonged exposure to CdTe QDs significantly decreased the expression levels of genes required for the synthesis and transport of GABA and cell identity of D-type motor neurons (Fig. 2E). Thus, prolonged exposure to low concentrations of CdTe QDs can cause adverse effects on both the development and the function of D-type motor neurons. Previous studies have also indicated the potential risk of prolonged or chronic exposure to QDs.[47] Our *in vivo* data further support the previous suggestions from *in vitro* data that QDs had neurotoxic effects.[12,13] Hence, we here provide direct evidence to raise a hypothesis that prolonged or chronic exposure to CdTe QDs can cause damage on both the development and the functions of nervous systems in animals.

In *C. elegans*, previous studies have shown the evidence that ENMs could be translocated into the reproductive organs, thereby causing damage to the reproductive behavior,[17,31,35,36,49] which suggests that the reproductive organs are important secondary targeted organs for ENMs in nematodes. Moreover, some previous studies have further demonstrated that ENMs exposure could cause damage on the functions of the nervous system,[31,33,34] indicating that neurons may also be the potential secondary targeted organs for ENMs in nematodes. However, there is still no direct evidence to support the role of neurons as the secondary targeted organ for ENMs in nematodes, to date In this study, along with the induction of shrinking behavior by CdTe QDs, we observed the distribution of CdTe QDs in the D-type motor neurons (Fig. 3), which demonstrates the potential of D-type motor neurons to act as the secondary targeted organs for CdTe QDs. Qu *et al.* and Hsu *et al.* have indicated that the translocation of QDs into the reproductive organs caused damage on the reproduction behavior.[35,36] Therefore, both reproductive organ and neurons are potential secondary targeted organs for QDs in nematodes.

As for the mechanism of neurotoxicity of CdTe QDs on D-type motor neurons, one possible explanation is that prolonged exposure to CdTe QDs may result in increased permeability of the intestine (primary targeted organ) and the further translocation of CdTe QDs into D-type motor neurons. Prolonged exposure to 1 μg L^{-1} of CdTe QDs caused the significant induction of intestinal ROS production (Fig. 4A and B) and the distribution of CdTe QDs throughout the body (Fig. 3). Another possible explanation is that CdTe QDs may cause neurodegeneration and dysfunction of D-type motor neurons following the translocation into D-type motor neurons. After prolonged exposure to CdTe QDs, ROS production could be induced in D-type motor neurons (Fig. 4C). Our data particularly showed that enhancement of cell identity of D-type motor neurons by overexpressing *unc-30* gene could effectively prevent the formation of shrinking behavior (Fig. 2F and G) and the toxic effects on the development of D-type motor neurons in CdTe QDs exposed nematodes (Fig. S1†). These results indicate the important role of cell identity in maintaining the functions of D-type motor neurons in CdTe QDs exposed nematodes. Moreover, strengthening the activity of Mn-SODs in D-type motor neurons also effectively inhibited the formation of shrinking behavior (Fig. 4F and G) and the adverse effects on the development of D-type motor neurons in CdTe QDs exposed nematodes (Fig. S1†). This supports the previous notion that activation of oxidative stress is a key mechanism of QDs-induced toxicity.[4–6]

In the present study, we further examined the underlying mechanism for the reduction of toxicity of CdTe QDs by ZnS coating. ZnS coating is an important method used to prevent toxicity on organisms from QDs.[35] Our data demonstrate that ZnS coating could effectively suppress the toxic effects of CdTe QDs exposure on the development and function of D-type motor neurons (Fig. 5). This is largely consistent with the pre-

vious study by Qu *et al.*, in which they used lifespan, growth, and brood size as the toxicity assessment endpoints.[35] Our results further suggest that the ZnS coating induced reduction in toxicity of CdTe QDs on the development and function of D-type motor neurons might be largely due to the blockage of QDs translocation into the D-type motor neurons (Fig. 5G). Moreover, we also found the decreased deposit of CdTe@ZnS QDs in intestine and the absence of induction of intestinal ROS production (Fig. 5G and H). These indicate that the defecation behavior may also contribute to the regulation of toxicity formation from CdTe QDs as suggested from studies on other ENMs in nematodes.[31,49] In rats, renal clearance is also an important mechanism for the reduction of QDs-induced toxicity.[50]

Conclusions

Our data provide the direct *in vivo* evidence indicating the toxic effects of CdTe QDs on the development and function of nervous system with the aid of D-type motor neurons as the assay system. In terms of the underlying mechanism of neurotoxicity from CdTe QDs, we hypothesize that the induction of CdTe QDs neurotoxicity may be due to both translocation into the targeted neurons and alterations in the development and function of the targeted neurons. In contrast, ZnS coating effectively suppressed the translocation of CdTe QDs into the targeted neurons so as to reduce the adverse effects on the development and function of targeted neurons. Considering the conserved property of GABAergic neurons between *C. elegans* and mammals, our data will shed light on our understanding of the potential risks of QDs on the development and function of nervous systems in other biological assay systems.

Conflict of interest

None of the authors have any conflicting interests.

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