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Exposure to low-dose nanopolystyrene induces the response of neuronal JNK MAPK signaling pathway in nematode *Caenorhabditis elegans*

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Abstract

Background: The response of organisms to nanoplastic exposure has gradually received the attention. Nevertheless, the role of neurons in response to nanoplastic exposure and the underlying mechanism are still largely unclear. We here examined the role of neuronal JNK MAPK signaling in response to low-dose of polystyrene (100 nm) in *Caenorhabditis elegans*.

Results: Exposure to nanopolystyrene in the range of $\mu\text{g/L}$ could increase the expression of genes (*jkk-1*, *mek-1*, and *jnk-1*) encoding JNK MAPK signaling pathway. Meanwhile, RNAi knockdown of any of these genes induced a susceptibility to nanopolystyrene toxicity. In the neurons, SNB-1/synaptobrevin was identified as the downstream target of JNK-1/JNK, suggesting the alteration in neurotransmitter signals in nanopolystyrene-exposed nematodes. In nanopolystyrene-exposed nematodes, JNK-1 modulated TBH-1-mediated octopamine signal and CAT-2-mediated dopamine signal. TBH-1 and CAT-2 further regulated the response to nanopolystyrene by affecting the function of corresponding intestinal octopamine receptors (SER-6 and OCTR-1) and intestinal dopamine receptor (DOP-1). In the intestine, DOP-1 regulated the response to nanopolystyrene by activating the downstream signaling cascade in p38 MAPK signaling pathway.

Conclusions: Exposure to low-dose of nanopolystyrene could induce the response of neuronal JNK MAPK signaling pathway in nematodes. Our data further highlight the crucial role of neuronal JNK MAPK signaling-activated alteration in octopamine and dopamine signals in regulating the response to nanopolystyrene in organisms.

Keywords: Nanopolystyrene, JNK MAPK signaling, Neuron, *Caenorhabditis elegans*

Background

A growing amount of reports have demonstrated that polymer microplastics (≤ 5 mm) have been frequently detected and widely distributed in different freshwater, marine, soil, and atmospheric environments, and even in the commodity salt [1–6]. Moreover, it has been found that the nanoplastics (≤ 100 nm), such as

nanopolystyrene, have been widely used in daily necessities, such as personal care products [7]. With the increase in production and use, a large amount of nanopolystyrene is potentially released into the environment. Meanwhile, microplastics in the real environment will also be gradually degraded into particles with nanosize [8]. Recently, the microplastics including nanoplastics have been frequently detected in Yangtze River basin, China [3, 9, 10]. Therefore, the toxicological responses of organisms to nanoplastics are needed to be examined.

The ecotoxicity at various aspects of nanopolystyrene exposure has been detected in different environmental

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organisms, such as monogonont rotifer and sea urchin [11–16]. Nanopolystyrene could not only be accumulated in exposed zebrafish, but also be transferred to the progeny [17]. *Caenorhabditis elegans* is a classic model animal, which was initially isolated from the soil environment [18]. *Caenorhabditis elegans* has been shown to be highly sensitive to the toxicity of environmental toxicants or stresses [19–21]. Some sublethal endpoints, such as brood size, locomotion behavior, and reactive oxygen species (ROS) production, have been raised for evaluating this sensitivity in nematodes [22–24]. *Caenorhabditis elegans* has been used for the assessment of both pristine and chemical modified nanopolystyrene particles in inducing toxicity at various aspects, such as reproductive toxicity and intestinal toxicity [25–28]. It has been predicted that the environmental concentrations of nanoplastics (100 nm) are in the range $\leq 1 \mu\text{g/L}$ [29, 30]. *Caenorhabditis elegans* is useful for detecting the potential toxicity of nanopolystyrene (100 nm) at predicted environmental concentration [31, 32]. In nematodes, some intestinal signaling pathways (such as insulin and p38 mitogen-activated protein kinase (MAPK) signaling pathways) are required for the response to nanopolystyrene [33–35]. Nevertheless, the roles of molecular signals in other tissues (such as neurons) in response to the nanopolystyrene are still largely unclear.

c-Jun N-terminal kinase (JNK) MAPK signaling pathway, one of the MAPK signaling family, acts as a central signaling hub to regulate various important biological processes by transducing extracellular cues into the cells [36, 37]. In nematodes, the JNK MAPK signaling has been also proven to be involved in the regulation of stress response [38, 39]. For example, the JNK MAPK signaling pathway was involved in the regulation of graphene oxide (GO) toxicity [39]. The *C. elegans* JNK MAPK signaling pathway mainly contains JNK-1, homolog of human JNK, and two MAP kinase kinases (MEK-1 and JKK-1) [40]. MEK-1 and JKK-1 act as activators of JNK [40]. *jnk-1* is expressed in the neurons [40]. In this study, we employed *C. elegans* as an animal model to investigate the response of neuronal JNK MAPK signaling to nanopolystyrene. Our results suggested the crucial role of JNK MAPK signaling pathway in response to nanopolystyrene. Moreover, our data highlight the important functions of octopamine and dopamine signals-mediated communication between neurons and intestine in regulating the response to nanopolystyrene in nematodes.

Materials and methods

Physicochemical characterizations of nanopolystyrene

Nanopolystyrene (100 nm) was from Janus New-Materials Co. (Nanjing, China). Working concentrations (0.1, 1, 10, and 100 $\mu\text{g/L}$) of nanopolystyrene were prepared

by diluting the stock solutions with liquid K-medium. Physicochemical properties of nanopolystyrene particles were examined by transmission electron microscopy (TEM, JEOL Ltd., Japan), Raman spectroscopy (Renishaw Invia Plus laser Raman spectrometer, Renishaw, UK), dynamic light scattering (DLS), and Zeta potential (Zetasizer Nano-ZS90, Malvern Instruments Ltd., UK). Before the use, the particle solutions were sonicated for 30 min (40 kHz, 100 W).

Animal maintenance

All nematodes were maintained on normal nematode growth media (NGM) agar plates fed with a lawn of *Escherichia coli* OP50 as the food source [18]. To collect synchronous L1 larvae, gravid nematodes were lysed with a bleaching mixture solution (0.45 M NaOH and 2% HOCl) to release eggs from the body of animals. After that, the eggs were transferred to a new NGM plates to let them develop into the L1 larvae.

Exposure and toxicity assessment

Nanopolystyrene exposure was performed from L1 larvae to adult day-3 in liquid solutions with the addition of OP50 ($\sim 4 \times 10^6$ colony-forming units (CFUs)) [33]. The nanopolystyrene solutions were refreshed daily.

ROS production was used to reflect the activation of oxidative stress in nanopolystyrene-exposed nematodes [41]. After the nanopolystyrene exposure, the nematodes were incubated with 1 μM CM- H_2DCFDA for 3 h without light. After the labeling, the nematodes were further washed with M9 buffer for three times. The nematodes were then transferred onto 2% agar pads for the examination at 488 nm of excitation wavelength and at 510 nm of emission filter with a laser scanning confocal microscope. In nematodes, the strongest ROS fluorescent signals can be detected in the intestine [42]. Fluorescence intensity in intestine was semi-quantified in comparison to the autofluorescence using Image J software. Fifty nematodes were examined per treatment.

Locomotion behaviors of head thrash and body bend were used as indicatives of functional state of motor neurons [43]. After the nanopolystyrene exposure, the nematodes were washed with M9 buffer for three times. To detect the number of head thrashes, 40 nematodes were randomly picked on the surface of an NGM plate without OP50 for the counting under a stereomicroscopy. A head thrash is defined as a change in the direction of bending at mid-body. Similarly, 40 nematodes were randomly picked onto an NGM plate without OP50 for the counting. A body bend is counted as a change in the direction of posterior bulb part along the y axis, assuming that the nematodes were traveling along the x axis.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of nematodes in each group was isolated using Trizol (Sigma-Aldrich, St. Louis, MO, USA). RNA purity and concentration were guaranteed by the ratio of OD₂₆₀/280 in a spectrophotometer. Mastercycler gradient PCR system (Eppendorf, USA) was employed to synthesize cDNA with reverse-transcriptase reaction. Expression levels of the examined genes were determined by StepOnePlus™ real-time PCR system (Applied Biosystems, Carlsbad, USA) with the SYBR Green qRT-PCR master mix (TOYOBO, Japan) and calculated using $2^{-\Delta\Delta C_t}$ method. The *tba-1* encoding alpha-tubulin protein was selected as a reference gene, and comparative cycle threshold methods were used to determine the relative quantities of the examined genes. Three biological replicates were performed in all reactions. Primers information for qRT-PCR is shown in Additional file 1: Table S1.

RNA interference (RNAi) assay

Before growing onto NGM agar plates, *E. coli* HT115 (DE3) was transferred into LA broth containing isopropyl 1-thio-β-D-galactopyranoside (IPTG, 5 mM). The L1 larvae were fed with HT115 (*E. coli* strain) carrying double-stranded RNA corresponding to *jkk-1*, *mek-1*, *jnk-1*, *snb-1*, *octr-1*, *tyra-3*, *ser-6*, *dop-1*, *tah-1*, or *cat-2* [44]. Once the L1 larvae on RNAi plates became the gravid animals, they were picked on fresh RNAi plate to lay eggs. The second generation was used for exposure and toxicity assessment. HT115 bacteria harboring empty vector L4440 was used as a control. RNAi efficiency was checked by qRT-PCR (Additional file 1: Figure S1).

DNA constructs and transformation

The promoter of *unc-14* (specially expressed in neurons) was amplified by PCR from wild-type nematode genomic DNA. PCR-amplified *jnk-1/B0478.1* was inserted into vector pPD_{95_77} carrying the *unc-14* promoter sequence. Germline transformation was conducted by co-injecting a testing DNA (1040 µg/mL) and a marker DNA of *Pdop-1::rfp* (60 µg/mL) into gonad [45]. Primers' information for vector constructions is shown in Additional file 1: Table S2.

Statistical analysis

Statistical analysis was carried out using SPSS Statistics 19.0 Software (SPSS Inc., USA.). After checking the normality using Agostino *D* test before parameters statistics, differences between groups were tested using one-way analysis of variance (ANOVA). Two-way

ANOVA analysis was further performed for multiple factor comparison. Probability level of 0.01 was considered statistically significant.

Results

Properties of nanopolystyrene

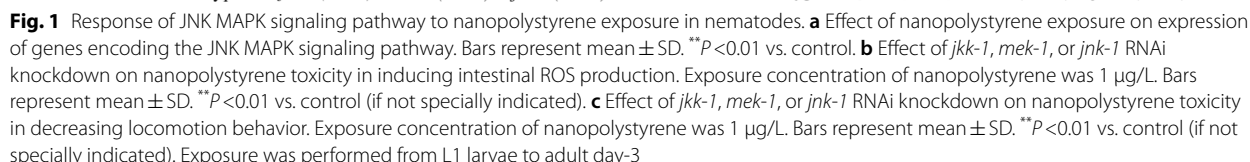
The TEM data show the morphology and the size of nanopolystyrene (Additional file 1: Figure S2a). After the sonication, the nanopolystyrene in the range of µg/L can be well dispersed in K medium [33]. The DLS analysis further confirmed that the size of nanopolystyrene after the sonication was 103.45 ± 4.8 nm. The Raman spectroscopy analysis indicated that the nanopolystyrene showed the peaks at 985.93 cm^{-1} (breathing vibration of benzene ring), at 1027.06 cm^{-1} and 1071.15 cm^{-1} (symmetric extension vibration of carbon atoms in benzene ring), at 1107.58 cm^{-1} , 1130.08 cm^{-1} , 1137.97 cm^{-1} , and 1146.82 cm^{-1} (stretching vibration of carbon atoms between benzene ring and polyethylene group), at 1350.26 cm^{-1} (asymmetric stretching vibration of carbon atoms between benzene ring and polyethylene group), at 1475.36 cm^{-1} (asymmetric bending vibration of carbon atoms and hydrogen atoms), and at 1513.10 cm^{-1} (asymmetric stretching vibration of benzene ring carbon atoms) (Additional file 1: Figure S2b). The zeta potential of nanopolystyrene was -9.213 ± 0.478 mV.

Effect of nanopolystyrene exposure on expressions of genes encoding JNK MAPK signaling pathway

After the exposure, nanopolystyrene (0.1 µg/L) did not affect the expressions of *jkk-1*, *mek-1*, and *jnk-1*, and nanopolystyrene (1 µg/L) also did not influence the expressions of *jkk-1* and *mek-1* (Fig. 1a). In contrast, exposure to nanopolystyrene (1 µg/L) significantly increased the *jnk-1* expression (Fig. 1a). Exposure to nanopolystyrene at concentrations of 10 and 100 µg/L further significantly increased the expressions of *jkk-1*, *mek-1*, and *jnk-1* (Fig. 1a).

RNAi knockdown of *jkk-1*, *mek-1*, or *jnk-1* induced a susceptibility to nanopolystyrene toxicity

Using intestinal ROS production and locomotion behavior as endpoints, we observed the more severe induction of intestinal ROS production and decrease in locomotion behavior in nanopolystyrene-exposed *jkk-1(RNAi)*, *mek-1(RNAi)*, or *jnk-1(RNAi)* nematodes compared with those in nanopolystyrene-exposed wild-type nematodes (Fig. 1b, c). That is, RNAi knockdown of *jkk-1*, *mek-1*, or *jnk-1* caused a susceptibility of nematodes to the nanopolystyrene toxicity.



To determine the genetic interaction between SNB-1 and JNK-1 in regulating the response to nanopolystyrene, we generated transgenic strain *Is(Punc-14-jnk-1)* overexpressing neuronal JNK-1. Neuronal overexpression of

JNK-1 could prevent the toxicity of nanopolystyrene in inducing intestinal ROS production and in decreasing locomotion behavior (Additional file 1: Figures S3d and S4b), suggesting the resistance of nematodes with neuronal overexpression of JNK-1 to nanopolystyrene toxicity. Moreover, we observed that RNAi knockdown of *snb-1* could induce the significant induction of intestinal ROS production and decrease in locomotion behavior in nanopolystyrene-exposed nematodes overexpressing neuronal JNK-1 (Additional file 1: Figures S3d and S4b). That is, RNAi knockdown of *snb-1* could suppress the resistance of nematodes overexpressing neuronal JNK-1 to nanopolystyrene toxicity. Therefore, SNB-1 acted as a downstream target of neuronal JNK-1 to regulate the response to nanopolystyrene.

Involvement of intestinal neurotransmitter receptors of OCTR-1, SER-6, and DOP-1 in regulating the response to nanopolystyrene

SNB-1/synaptobrevin is a vesicle-associated protein, which mediates the biological process of neurotransmitter release [48]. After the exposure, a large amount of nanopolystyrene particles were translocated and accumulated in the intestinal cells [49]. We assumed that the SNB-1-mediated neuronal neurotransmission may

regulate the nanopolystyrene toxicity by activating the corresponding intestinal neurotransmitter receptors. Among the neurotransmitter receptors in nematodes, 2 octopamine receptors (OCTR-1 and SER-6), 1 tyramine receptor (TYRA-3), 3 serotonin receptors (SER-1, SER-3, and SER-5), 3 acetylcholine receptors (ACR-9, ACR-14, and ACR-17), and 2 dopamine receptors (DOP-1 and DOP-4) can be expressed in the intestinal cells (Additional file 1: Table S3). Among the genes encoding these intestinal neurotransmitter receptors, exposure to nanopolystyrene could significantly decrease the expression of *octr-1* and increase the expressions of *tyra-3*, *ser-6*, and *dop-1* (Fig. 2a). Using VP303/*rde-1(ne219);kbls7[nhx-2p::rde-1 + rol-6(su1006)]* as a genetic tool for intestine-specific RNAi knockdown of gene(s) [50], we found that intestine-specific RNAi knockdown of *tyra-3* did not significantly affect the nanopolystyrene toxicity in inducing intestinal ROS production (Fig. 2b). In contrast, intestine-specific RNAi knockdown of *octr-1* suppressed the nanopolystyrene toxicity in inducing intestinal ROS production, and intestine-specific RNAi knockdown of *ser-6* or *dop-1* caused the more severe induction of intestinal ROS production in nanopolystyrene-exposed nematodes compared with that in nanopolystyrene-exposed VP303 nematodes (Fig. 2b). These observations suggested that

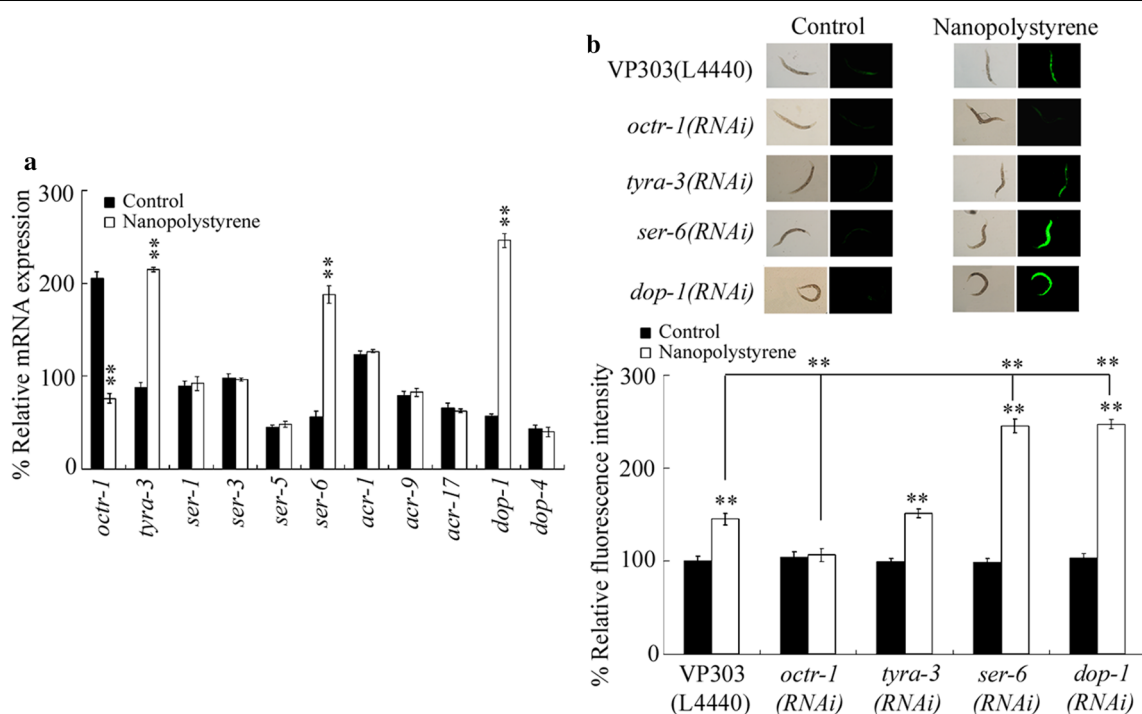


Fig. 2 Identification of intestinal neurotransmitter receptors involved in the regulation of response to nanopolystyrene. **a** Effect of nanopolystyrene exposure on expressions of genes encoding intestinal neurotransmitter receptors. Bars represent mean \pm SD. ** $P < 0.01$ vs. control. **b** Effect of *octr-1*, *tyra-3*, *ser-6*, or *dop-1* RNAi knockdown on nanopolystyrene toxicity in inducing intestinal ROS production. Bars represent mean \pm SD. ** $P < 0.01$ vs. control (if not specially indicated). Exposure concentration of nanopolystyrene was 1 μ g/L. Exposure was performed from L1 larvae to adult day-3

intestinal octopamine receptors (OCTR-1 and SER-6) and dopamine receptor (DOP-1) were involved in the control of response to nanopolystyrene.

Involvement of TBH-1 and CAT-2 in the control of response to nanopolystyrene

In nematodes, the octopamine biosynthesis requires tyramine β -hydroxylase TBH-1 to convert tyramine into octopamine [51], and the dopamine biosynthesis requires tyrosine hydroxylase CAT-2 [52]. Nanopolystyrene exposure could significantly increase the *tbh-1* expression and decrease the *cat-2* expression (Fig. 3a). Meanwhile, RNAi knockdown of *jnk-1* could cause the

significant decrease in *tbh-1* expression and increase in *cat-2* expression in nanopolystyrene-exposed nematodes (Fig. 3b). Moreover, we observed the more severe induction of intestinal ROS production and decrease in locomotion behavior in nanopolystyrene-exposed *tbh-1(RNAi)* nematodes compared with nanopolystyrene-exposed wild-type nematodes (Fig. 3c, d). In contrast, RNAi knockdown of *cat-2* significantly inhibited the nanopolystyrene toxicity in inducing intestinal ROS production and in decreasing locomotion behavior (Fig. 3c, d). Therefore, both TBH-1 and CAT-2 were involved in the regulation of response to nanopolystyrene.

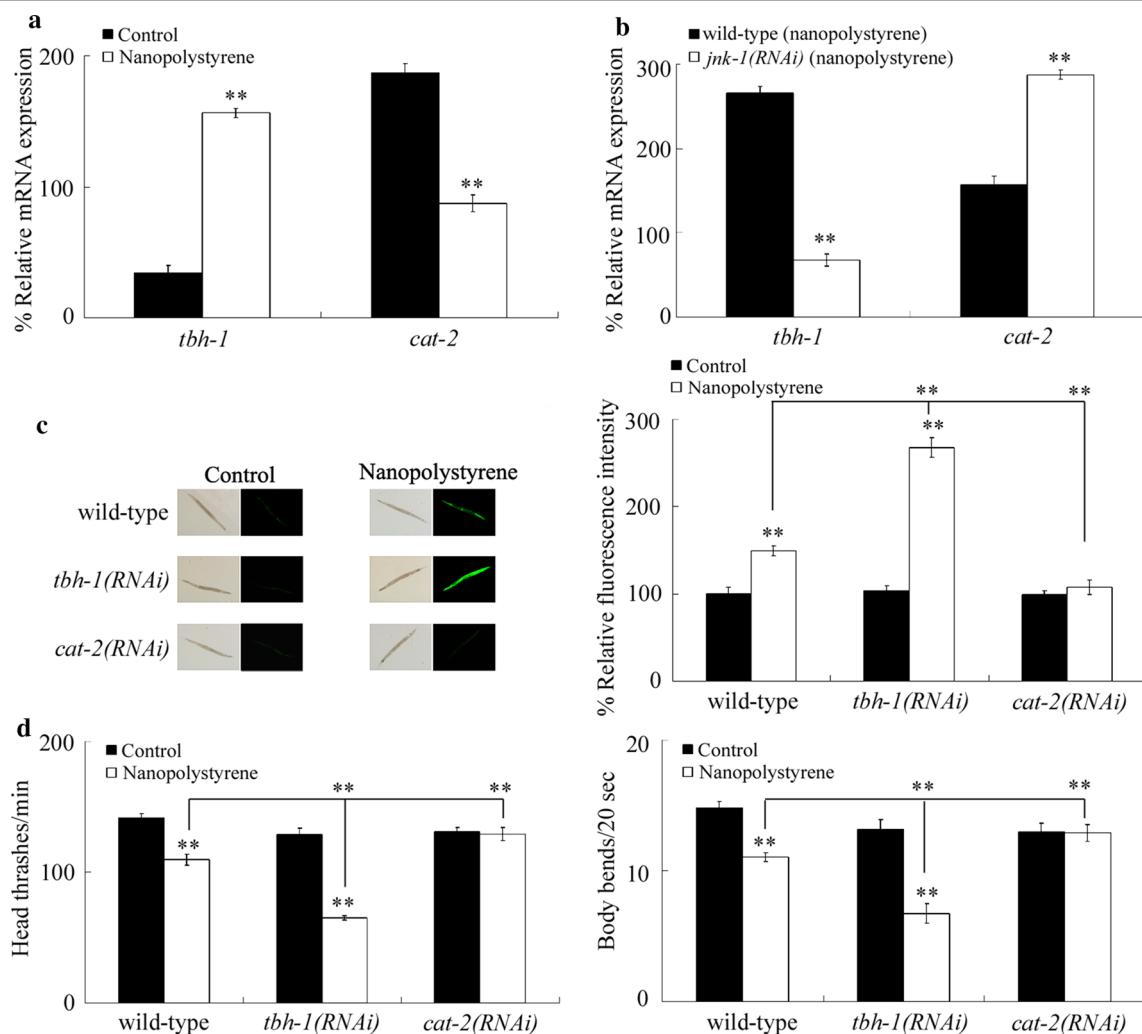


Fig. 3 Involvement of TBH-1 and CAT-2 in the control of response to nanopolystyrene. **a** Effect of nanopolystyrene exposure on expressions of *tbh-1* and *cat-2*. Bars represent mean \pm SD. ** P < 0.01 vs. control. **b** Effect of *jnk-1* RNAi knockdown on expressions of *tbh-1* and *cat-2* in nanopolystyrene-exposed nematodes. Bars represent mean \pm SD. ** P < 0.01 vs. wild-type. **c** Effect of *tbh-1* or *cat-2* RNAi knockdown on nanopolystyrene toxicity in inducing intestinal ROS production. Bars represent mean \pm SD. ** P < 0.01 vs. control (if not specially indicated). **d** Effect of *tbh-1* or *cat-2* RNAi knockdown on nanopolystyrene toxicity in decreasing locomotion behavior. Bars represent mean \pm SD. ** P < 0.01 vs. control (if not specially indicated). Exposure concentration of nanopolystyrene was 1 μ g/L. Exposure was performed from L1 larvae to adult day-3

Genetic interaction between JNK-1 and TBH-1 or CAT-2 in regulating the response to nanopolystyrene

To determine the genetic interaction between JNK-1 and TBH-1 in regulating the response to nanopolystyrene, we performed RNAi knockdown of *tbh-1* in nematodes overexpressing neuronal JNK-1. We observed that RNAi knockdown of *tbh-1* could induce the significant ROS production and decrease in locomotion behavior in nanopolystyrene-exposed nematodes overexpressing neuronal JNK-1 (Additional file 1: Figure S5), which suggested that RNAi knockdown of *tbh-1* could suppress the resistance of nematodes overexpressing neuronal JNK-1 to nanopolystyrene toxicity. Meanwhile, we observed that RNAi knockdown of *cat-2* inhibited the induction of ROS production and the decrease in locomotion behavior in nanopolystyrene-exposed *jnk-1(RNAi)* nematodes (Additional file 1: Figure S5), which suggested that RNAi knockdown of *cat-2* could suppress the susceptibility of *jnk-1(RNAi)* nematodes to nanopolystyrene toxicity.

Effect of RNAi knockdown of *cat-2* or *tbh-1* on expression of intestinal genes encoding the corresponding neurotransmitter receptors in nanopolystyrene-exposed nematodes

To determine the octopamine- and the dopamine-mediated communication between neurons and intestine, we next examined the effect of RNAi knockdown of *cat-2* or *tbh-1* on expression of intestinal genes encoding the corresponding neurotransmitter receptors in nanopolystyrene-exposed nematodes. In nanopolystyrene-exposed nematodes, RNAi knockdown of *cat-2* could significantly increase the expression of intestinal *dop-1* (Fig. 4a). Meanwhile, in nanopolystyrene-exposed nematodes, RNAi knockdown of *tbh-1* could significantly decrease the expression of intestinal *ser-6* and increase the expression of intestinal *octr-1* (Fig. 4b).

Effect of intestinal RNAi knockdown of *dop-1*, *octr-1*, or *ser-6* on expression of genes encoding p38 MAPK and insulin signaling pathways in nanopolystyrene-exposed nematodes

Our previous studies have indicated that p38 MAPK and insulin signaling pathways acted in the intestine to regulate the response to nanopolystyrene [33, 34]. In nematodes, *pmk-1* encodes the p38 MAPK in p38 MAPK signaling pathway, and *daf-16* encodes the FOXO transcriptional factor in insulin signaling pathway. Recently, it has been further shown that the signaling cascade of *mdt-15-sbp-1* encoding the lipid metabolic signaling acted downstream of p38 MAPK signaling to regulate the response to nanopolystyrene [35]. In the intestine, we further examined the effect of RNAi knockdown of *dop-1*, *octr-1*, or *ser-6* on expression of genes encoding p38

MAPK and insulin signaling pathways in nanopolystyrene-exposed nematodes. In nanopolystyrene-exposed nematodes, intestinal RNAi knockdown of *dop-1* could significantly decrease the expressions of *pmk-1*, *mdt-15*, and *sbp-1* (Fig. 4c). In contrast, in nanopolystyrene-exposed nematodes, intestinal RNAi knockdown of *octr-1* or *ser-6* did not affect the expressions of *pmk-1*, *mdt-15*, and *sbp-1* (Fig. 4c). In addition, in nanopolystyrene-exposed nematodes, intestinal RNAi knockdown of *dop-1*, *octr-1* or *ser-6* did not influence the expression of *daf-16* (Fig. 4d).

Discussion

In nematodes, MEK-1 and JKK-1 act as co-activators of JNK/JNK-1 [21, 24, 40]. In this study, we observed that exposure to nanopolystyrene (100 nm) in the range of $\mu\text{g/L}$ could significantly increase the expressions of *jdk-1*, *mek-1*, and *jnk-1* (Fig. 1a). Meanwhile, using intestinal ROS production and locomotion behavior as the endpoints, RNAi knockdown of *jdk-1*, *mek-1*, or *jnk-1* induced a susceptibility to nanopolystyrene toxicity (Fig. 1b, c). Therefore, the alteration in genes encoding the JNK MAPK signaling pathway induced by nanopolystyrene in the range of $\mu\text{g/L}$ mediated a protective response to nanopolystyrene. Similarly, the alteration in genes encoding insulin and p38 MAPK signaling pathways induced by nanopolystyrene in the range of $\mu\text{g/L}$ also mediated a protective response to nanopolystyrene in nematodes [33–35].

Some reports have predicted that the range of environmental concentrations for nanoplastics (1–100 nm) is $\leq 1 \mu\text{g/L}$ [29, 30]. In this study, we observed that exposure to 100 nm nanopolystyrene (1 $\mu\text{g/L}$) could only increase the *jnk-1* expression in JNK MAPK signaling pathway (Fig. 1a). Our previous study has also indicated that exposure to 100 nm nanopolystyrene (1 $\mu\text{g/L}$) could only increase the *pmk-1* expression in p38 MAPK signaling pathway [34]. That is, exposure to nanopolystyrene at predicted environmental concentration may only be able to affect the expression of gene encoding MAPK in JNK MAPK or p38 MAPK signaling pathway in nematodes.

Considering the fact that *jnk-1* is expressed in the neurons [21, 24, 40], the JNK MAPK signaling provided an important molecular basis for neuronal response to nanopolystyrene. In *C. elegans*, there are three MAPK signaling pathways (p38, JNK, and ERK signaling pathways) [21, 24]. p38 MAPK signaling pathway acted in the intestine to regulate the response to nanopolystyrene in nematodes [34, 35]. These observations suggest that different MAPK signaling pathways can provide the molecular basis for different tissues in response to nanopolystyrene.

In the neurons, SNB-1/synaptobrevin was identified as the downstream target of JNK-1/JNK during the

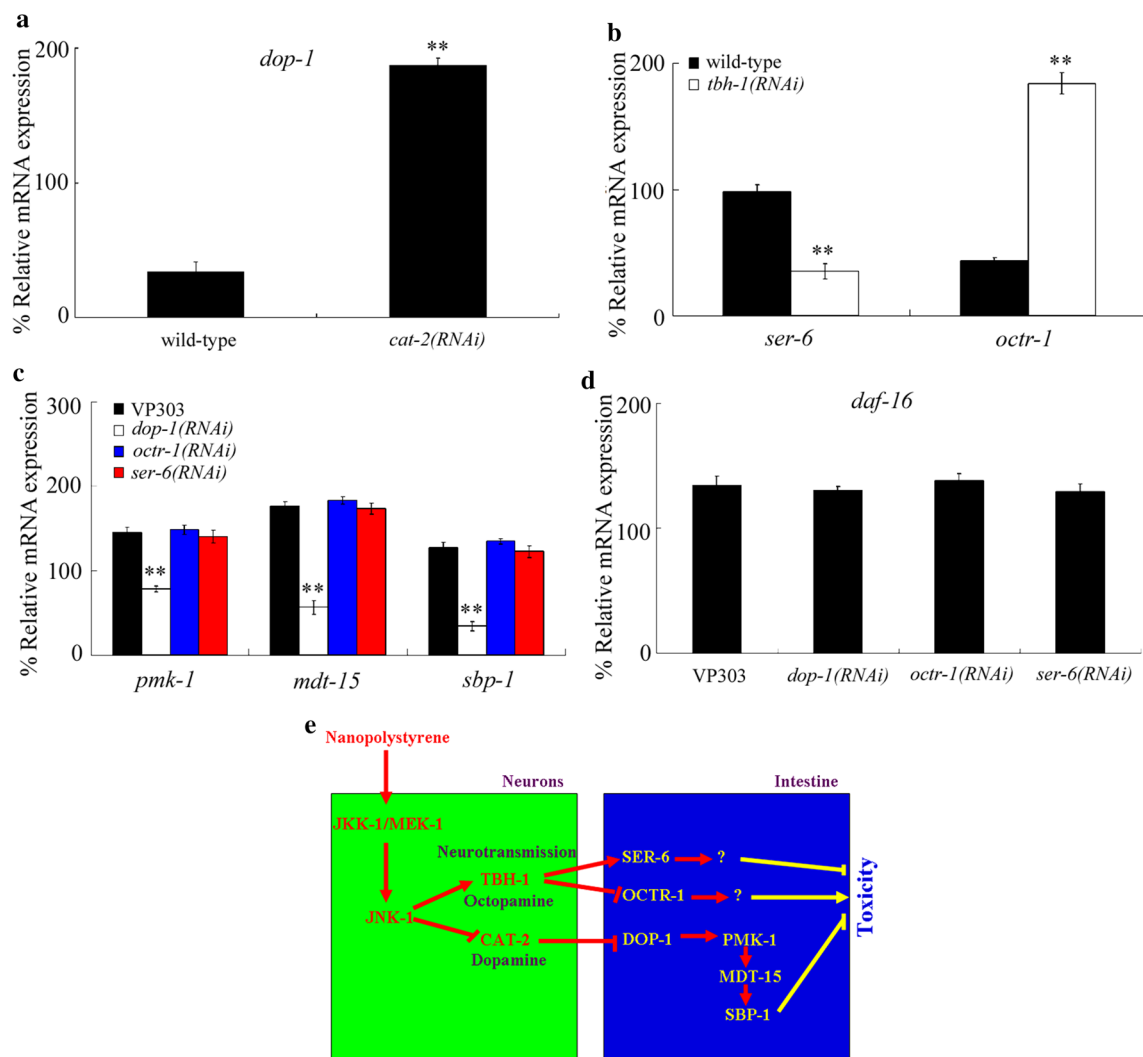


Fig. 4 Involvement of octopamine and dopamine signals in the regulation of response to nanopolystyrene. **a** Effect of RNAi knockdown of *cat-2* on expression of intestinal *dop-1* in nanopolystyrene-exposed nematodes. qRT-PCR was performed in isolated intact intestines ($n=40$). Exposure concentration of nanopolystyrene was 1 $\mu\text{g/L}$. Exposure was performed from L1 larvae to adult day-3. Bars represent mean \pm SD. ** $P<0.01$ vs. wild-type. **b** Effect of RNAi knockdown of *tbh-1* on expressions of intestinal *ser-6* and *octr-1* in nanopolystyrene-exposed nematodes. qRT-PCR was performed in isolated intact intestines ($n=40$). Exposure concentration of nanopolystyrene was 1 $\mu\text{g/L}$. Exposure was performed from L1 larvae to adult day-3. Bars represent mean \pm SD. ** $P<0.01$ vs. wild-type. **c** Effect of intestinal RNAi knockdown of *dop-1*, *octr-1*, or *ser-6* on expressions of *pmk-1*, *mdt-15*, or *sbp-1* in nanopolystyrene-exposed nematodes. Exposure concentration of nanopolystyrene was 1 $\mu\text{g/L}$. Exposure was performed from L1 larvae to adult day-3. Bars represent mean \pm SD. ** $P<0.01$ vs. VP303. **d** Effect of intestinal RNAi knockdown of *dop-1*, *octr-1*, or *ser-6* on expression of *daf-16* in nanopolystyrene-exposed nematodes. Exposure concentration of nanopolystyrene was 1 $\mu\text{g/L}$. Exposure was performed from L1 larvae to adult day-3. Bars represent mean \pm SD. ** $P<0.01$ vs. VP303. **e** A diagram showing the molecular basis of neuronal JNK MAPK signaling in regulating the response to nanopolystyrene in nematodes

regulation of response to nanopolystyrene (Additional file 1: Figure S3). The vesicle-associated protein SNB-1 mediates the neurotransmitter release [48], which suggested that the JNK MAPK signaling may regulate the response to nanopolystyrene by potentially modulating the neurotransmitter signals in the neurons (Fig. 4e). In nematodes, it was also reported that the PKC-1 governing the neurotransmitter release also acted as the

downstream target of neuronal NLG-1 signaling to regulate the response to GO [21, 24, 53]. In the neurons, we further provide the evidence to indicate the role of JNK MAPK signaling in modulating the TBH-1-mediated octopamine signal and the CAT-2-mediated dopamine signal in nanopolystyrene-exposed nematodes (Fig. 3 and Additional file 1: Figure S5), which supported the function of neuronal JNK MAPK signaling raised above.

Nevertheless, the identification of TBH-1-mediated octopamine signal and CAT-2-mediated dopamine signal in nanopolystyrene-exposed nematodes was based on the JNK MAPK signaling-activated communication between the neurons and the intestine. That is, we still do not exclude the possibility that the other possible neurotransmitter signals may also be modulated by neuronal JNK MAPK signaling in nanopolystyrene-exposed nematodes.

In nematodes, TBH-1 and CAT-2 regulated the response to nanopolystyrene by affecting the functions of corresponding intestinal octopamine receptors (SER-6 and OCTR-1) and intestinal dopamine receptor (DOP-1) (Figs. 2 and 4a, b). Especially, RNAi knockdown of *tbh-1* could decrease the *ser-6* expression and increase the *octr-1* expression in nanopolystyrene-exposed nematodes (Fig. 4b), which suggested that the neuronal TBH-1 can activate both positive and negative regulators in the intestine to regulate the response to nanopolystyrene. These observations demonstrated that the neuronal TBH-1 and CAT-2 and their corresponding intestinal octopamine and dopamine receptors established a communication between the neurons and the intestine required for the control of response to nanopolystyrene in nematodes (Fig. 4e). These observations suggested an important molecular basis for the communication between the neurons and the intestine in nanopolystyrene-exposed nematodes.

So far, in the intestine, two signaling pathways (insulin and p38 MAPK signaling pathways) have been identified to be involved in the control of response to nanopolystyrene [33–35, 54]. In the intestine, we found that the DOP-1 regulated the response to nanopolystyrene by activating the downstream signaling cascade in p38 MAPK signaling pathway (Fig. 4c). The downstream targets of SER-6 and OCTR-1 in the intestine during the control of response to nanopolystyrene are still unclear (Fig. 4e). Meanwhile, we found that DOP-1, SER-6, and OCTR-1 were not the upregulators of intestinal DAF-16 in the insulin signaling pathway in the regulation of response to nanopolystyrene (Fig. 4d). In the intestine, the insulin receptor DAF-2 could regulate the response to nanopolystyrene by suppressing the function of DAF-16 in nematodes [33].

Conclusions

We here employed *C. elegans* as an animal model to investigate the role of neuronal JNK MAPK signaling in response to nanopolystyrene and the underlying mechanism. In nematodes, exposure to 100 nm nanopolystyrene in the range of $\mu\text{g/L}$ induced the increase of genes encoding JNK MAPK signaling pathway, which mediated a protective response to nanopolystyrene. This activated JNK MAPK signaling modulated the TBH-1-mediated

octopamine and CAT-2-mediated dopamine signals in neurons in nanopolystyrene-exposed nematodes. TBH-1 regulated the response to nanopolystyrene by affecting its intestinal receptors of SER-6 and OCTR-1, and CAT-2 regulated the response to nanopolystyrene by affecting its intestinal receptor of DOP-1. In the intestine, dopamine receptor further regulated the response to nanopolystyrene by activating the p38 MAPK signaling pathway. Our results highlight the importance of octopamine- and dopamine-mediated neuron–intestine communication activated by neuronal JNK MAPK signaling in regulating the response to nanopolystyrene in organisms.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12302-020-00331-8>.

Additional file 1: Figure S1. RNAi efficiency confirmed by qRT-PCR. L4440, empty vector. Bars represent mean \pm SD. ** $P < 0.01$ vs. L4440. **Figure S2.** Properties of nanopolystyrene. **Figure S3.** Identification of targets of JNK-1 in regulating the response to nanopolystyrene. **Figure S4.** Effect of *snb-1* RNAi knockdown on nanopolystyrene toxicity in nematodes over-expressing neuronal JNK-1. **Figure S5.** Genetic interaction between JNK-1 and TBH-1 or CAT-2 in regulating the nanopolystyrene toxicity in inducing intestinal ROS production. **Table S1.** Primer information for qRT-PCR. **Table S2.** Primer information for vector constructions.

Abbreviations

ROS: Reactive oxygen species; MAPK: Mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; TEM: Transmission electron microscopy; DLS: Dynamic light scattering; CFUs: Colony-forming units; NGM: Nematode growth media; qRT-PCR: Quantitative real-time polymerase chain reaction; RNAi: RNA interference; IPTG: Isopropyl 1-thio- β -D-galactopyranoside; ANOVA: Analysis of variance.

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Authors' contributions

MQ, DL, YZ, and YY performed the experiments. DW designed the experiment and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article and supporting materials.

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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