The effects of NONRATT021972 lncRNA siRNA on PC12 neuronal injury mediated by P2X₇ receptor after exposure to oxygen-glucose deprivation

Guilin Li¹ · Lifang Zou¹ · Wei Xie² · Shiyao Wen² · Qiuyu Xie² · Yun Gao¹ · Changshui Xu¹ · Hong Xu¹ · Shuangmei Liu¹ · Shouyu Wang¹ · Yun Xue¹ · Bing Wu¹ · Qiulan Lv¹ · Mofeng Ying¹ · Xi Zhang¹ · Shangdong Liang¹

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Abstract Adenosine triphosphate (ATP) participates in signal transmission by acting on P2X receptors, and the P2X₇ receptor is involved in the pathophysiological changes of ischemic injury. The PC12 cell line is a popular model system to study sympathetic neuronal function. Long non-coding RNAs (lncRNAs) are highly expressed in the nervous system and serve as regulatory RNAs. In this study, the effects of NONRATT021972 lncRNA siRNA on P2X₇-mediated PC12 neuronal injury after exposure to oxygen-glucose deprivation (OGD) were investigated. Our results showed that the viability of PC12 cells cultured with OGD or the P2X₇ agonist BzATP was significantly decreased. Treatment with NONRATT021972 siRNA reversed the decreased viability of PC12 cells under OGD conditions. The upregulated P2X₇ mRNA and protein levels in PC12 cells under OGD conditions or BzATP treatment were significantly decreased when pretreated with NONRATT021972 siRNA. Moreover, NONRATT021972 siRNA treatment effectively suppressed the increase in [Ca²⁺]_i induced by OGD or P2X₇ agonists (ATP or BzATP) in PC12 cells. Therefore, treatment with NONRATT021972 siRNA may decrease sympathetic neuronal injury induced by ischemia.

Keywords PC12 cell · Adenosine triphosphate · P2X₇ receptor · Long noncoding RNA · Oxygen-glucose deprivation · Intracellular Ca²⁺

Introduction

Extracellular purines (adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP)) and pyrimidines (UTP and UDP) act through the plasma membrane of P2 receptors. P2 receptors are classified into P2X ionotropic ligand-gated ion channel receptors (P2X₁–P2X₇) and P2Y metabotropic G-protein-coupled receptors (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, and P2Y₁₄) [1–3]. ATP is the native agonist for P2X receptors. After ATP binding to P2X receptors, the pore permeable to Na⁺, K⁺, and Ca²⁺ is opened. The rat pheochromocytoma cell line, PC12, has been reported to express P2 receptors and respond to purinergic agonists through P2 receptors [4–6]. The P2X₇ receptor is a member of the P2X family, which plays an important role in the transmission of inflammatory and nociceptive signals [2, 7–11].

Sympathetic ganglia have been implicated in ischemic heart diseases [12–21]. In myocardial ischemic injury, ATP released from damaged cells activates P2X receptors in sympathetic afferent and efferent nerves [2, 7, 12–18, 21–23]. Moreover, application of exogenous ATP results in neuronal injury by excitotoxic action [2, 7, 15, 16, 19, 20, 24]. Oxygen-glucose deprivation (OGD) can occur in ischemia, and it is related to neuronal injury [7, 25, 26]. The P2X₇ receptor is involved in neuronal injury after ischemic diseases [7, 17, 21, 27].

Long noncoding RNAs (lncRNAs) are a large subgroup of the mammalian transcriptome and are highly expressed in the nervous system [28]. lncRNAs serve

Guilin Li and Lifang Zou are both first authors.

✉ Shangdong Liang
liangsd@hotmail.com

¹ Department of Physiology, Medical School of Nanchang University, Nanchang 330006, People's Republic of China

² Undergraduate student of grade 2012, Medical School of Nanchang University, Nanchang 330006, People's Republic of China

as regulatory RNAs, which can affect mRNA transcription, splicing, export, stability, and translation [29, 30]. Genetic knockout of some lncRNAs in mice results in perinatal lethality, postnatal lethality, or developmental defects [30]. Therefore, some lncRNAs participate in physiological processes that maintain cellular and tissue homeostasis, indicating that the dysregulated expression of lncRNAs contributes to the onset and progression of many diseases [28, 31, 32]. The roles of lncRNAs in the sympathetic nervous system remain poorly characterized. NONRATT021972 is an lncRNA [<http://www.noncode.org>; 33]. Our previous studies have shown that the P2X₇ receptor is implicated in the pathological injury of sympathetic nerves [17, 18, 21, 24, 34, 35]. Although lncRNAs are highly expressed in the brain, their roles in the peripheral nervous system are still unclear. The aim of this research was to investigate the effects of NONRATT021972 small interference RNA (siRNA) on PC12 neuronal injury mediated by the P2X₇ receptor after exposure to OGD.

Materials and methods

Cell cultures

The PC12 cell line was originally obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). PC12 cells were seeded in 25-cm² polystyrene flasks in Dulbecco's modified Eagle's medium (DMEM) (high glucose) (Hyclone, USA) containing 5 % heat-inactivated fetal bovine serum (Gibco, USA) and 10 % horse serum (Gibco, USA). The cells were incubated under a humidified atmosphere containing 5 % CO₂ (normal culture incubator) at 37 °C, and the culture medium was replaced every 48 h. PC12 cells were plated in six-well plates containing 2 mL of complete medium at a density of 1.0×10^5 /mL cells. When cell density reached 70–80 %, we established a model for oxygen glucose deprivation (OGD) [25].

Drugs and groups

ATP (100 μM, Sigma), BzATP (30 μM, Sigma), and BBG (100 nM, Sigma) were used. According to the experimental procedure, PC12 cells were randomly divided into the following groups: (1) control group; (2) oxygen-glucose deprivation (OGD) group; (3) OGD+scramble siRNA (OGD+NC siRNA) group; (4) OGD+NONRATT021972 siRNA (OGD+lnc siRNA) group; (5) OGD+BzATP group; and (6) OGD+brilliant blue G (BBG) group. The OGD+scramble siRNA group was only used for testing the interference efficiency of NONRATT021972 siRNA.

Oxygen glucose deprivation (OGD)

For OGD treatment [25, 36, 37], the culture medium were changed to a deoxygenated glucose-free Earle's balanced salt solution at pH 7.4, and 10 % fetal bovine serum was added. The cells were then transferred to a sealed hypoxic box containing a mixture of 95 % N₂ and 5 % CO₂ at 37 °C for 2 h. After OGD, the cells were cultured in full culture medium with oxygen for 24 h and treated with the indicated drugs. Oxygenated glucose-containing Earle's balanced salt solution was used as control.

MTS analysis of cell viability

PC12 cells were seeded into 96-well tissue culture plates with 0.1-mL culture medium at a density of 1×10^5 cells/mL. After growth for 48 h, the cells were treated with OGD for 2 h. After reperfusion for 48 h, cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay [38]. Each group contained ten samples, and 20 μl of a mixture of MTS and phenazine methosulfate (Promega) was pipetted into each well of the 96-well assay plate containing 100 μl of fetal bovine serum-free medium. The plate was incubated at 37 °C for 3 h in a humidified atmosphere with 5 % CO₂. Absorbance was detected using a microplate reader (Rayto RT-6000, Ruixin) at 490 nm. Background absorbance was subtracted using a set of wells containing medium only.

siRNA knockdown experiment

Small interfering RNA (siRNA) technology was used to knockdown NONRATT021972 lncRNA in OGD PC12 cells [36, 37]. Three different siRNAs targeting specific sequences of NONRATT021972 and a negative control scrambled siRNA (not homologous to any gene) were synthesized by RiboBio Co. Ltd. (Guangzhou, China). The optimal NONRATT021972 siRNA was selected based on real-time RT-PCR analysis (data not shown). The sequences of the optimal siRNA were as follows: sense 5'-GAA UGU UGG UCA UAU CAA AdTdT-3' and antisense 3'-dTdT CUU ACA ACC AGU AUA GUU U-5'. The NONRATT021972 siRNA fragment was transfected into cultured cells by Lipofectamine 2000 (Invitrogen). In detail, the NONRATT021972 siRNA and Lipofectamine 2000 were diluted in Opti-MEM[®] medium without serum, and the final concentration of NONRATT021972 siRNA was 50 nM.

Measurement of intracellular calcium concentration

Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured with the fluorescent Ca²⁺-sensitive dye, Fura 2-acetoxymethyl ester (fura-2/AM), as described previously

[24]. PC12 cells were divided into the following groups: control group, OGD group, OGD+NC siRNA group, OGD+NONRATT021972 siRNA group, OGD+BzATP group, and OGD+BBG group. PC12 cells cultured in OGD conditions were seeded in six-well plates and incubated with NONRATT021972 siRNA (50 nM), BzATP (30 μ M), or BBG (100 nM) [39, 40]. PC12 cells in six-well plates were loaded with fura-2/AM at a final concentration of 1 mol/L. The cells were then incubated under the indicated conditions. Aliquots of cell suspensions were centrifuged and added to a cuvette on a spectrofluorometer (Perkin-Elmer LS-50B; Perkin-Elmer, Waltham, MA, USA). The fura-2-generated fluorescence was monitored and recorded by ratio fluorometry with an emission wavelength of 510 nm at alternate 340/380-nm excitation wavelengths. The fluorescence ratio (F340/F380) was calculated as an indicator of $[Ca^{2+}]_i$.

Real-time RT-PCR

Total RNA of PC12 cells cultured under different conditions was isolated by TRIzol Total RNA Reagent (Beijing Tiangen Biotech Co.). The reverse transcription reaction was completed using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Glen Bernie, USA) following the manufacturer's instructions. The following primers were designed using Primer Express 3.0 software (Applied Biosystems): 5'-TCA GAT CGG TGC ATC AAA GC-3' (forward) and 5'-GGA CTG GGC AGC AGG AAT GA-3' (reverse) for NONRATT021972; 5'-CTT CGG CGT GCG TTT TG-3' (forward) and 5'-AGG ACA GGG TGG ATC CAA TG-3' (reverse) for P2X₇; and 5'-CAC CCG CGA GTA CAA CCT TC-3' (forward) and 5'-CCC ATA CCC ACC ATC ACA CC-3' (reverse) for β -actin. Quantitative PCR was performed using the SYBR® Green MasterMix in an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems Inc., Foster City, CA). The quantification of gene expression was performed using the $\Delta\Delta CT$ calculation with CT as the threshold cycle. The relative levels of target genes, which were normalized to the sample with the lowest CT, were given as $2^{-\Delta\Delta CT}$ [41].

Western blotting

The level of P2X₇ protein was determined by western blotting. The cells were solubilized in lysis buffer. After incubating on ice for 30 min, the lysates were clarified by centrifugation at 14,000g for 15 min. The supernatant was removed, and the protein concentration was determined using the Bradford protein assay system (Bio-Rad). Samples containing equal amounts of protein (20 μ g) were separated by SDS-polyacrylamide (10 %) gel electrophoresis and electrophoretically transferred onto Immobilon polyvinylidene difluoride membranes. After blocked with 5 % nonfat dry milk, the

membranes were incubated with the following primary antibodies: monoclonal anti-P2X₇ (1:1000) and anti- β -actin (1:1000). The membranes were washed and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (diluted 1:5000, Beijing Zhongshan Biotechnology Co.). Chemiluminescent signals were collected on autoradiography film using the enhanced chemiluminescence (ECL) kit (Shanghai Pufei Biotechnology Co.). The band intensities were quantified using Image Pro-Plus software. Protein expression levels were represented as densitometric ratios of the targeted protein to β -actin.

Statistical analysis

All experiments were performed in triplicate, and the data were presented as the means $\pm$ standard error of the mean (SEM). All statistical analyses were performed using SPSS 17.0 software. Statistical significance was assessed using the one-way analysis of variance for multiple group comparisons followed by Tukey's post hoc test. *p* Values less than 0.05 were considered to be statistically significant.

Results

Effects of OGD on PC12 cell growth and NONRATT021972 expression

The growth of PC12 cells cultured with or without OGD was observed (Fig. 1a, b). Morphological observation indicated that PC12 cells cultured in normal conditions displayed larger, shinier, and fuller perikaryon as well as longer synapses, but cells subjected to OGD showed slender perikaryon and shorter synapses. The expression of NONRATT021972 lncRNA in PC12 cells was evaluated by real-time RT-PCR. The expression of NONRATT021972 lncRNA in PC12 cells subjected to OGD was 2.8-fold higher than that in cells cultured in control medium ($p < 0.01$; Fig. 1c).

Effects of NONRATT021972 siRNA on the viability of PC12 cells after OGD treatment

To investigate the effects of NONRATT021972 siRNA on OGD-induced cell viability in PC12 cells, NONRATT021972 siRNA was transfected into PC12 cells cultured with OGD. PC12 cells were cultured in control or OGD conditions, and cells cultured in OGD conditions were also treated with scramble siRNA (OGD+NCsiRNA), NONRATT021972 siRNA (OGD+lncsiRNA), P2X₇ agonist (30 μ M BzATP, OGD+BzATP), or P2X₇ antagonist (100 nM BBG, OGD+BBG) for 48 h. The MTS assay was used to assess the effects of NONRATT021972 lncRNA knockdown on the

Fig. 1 The effects of OGD on the growth of PC12 cells and the expression of NONRATT021972 lncRNA (c). PC12 cells cultured in the control group (a) and OGD group (b) are indicated by arrows. PC12 cells cultured in the control group showed larger, shinier, and fuller perikaryon as well as longer synapses. PC12 cells cultured under OGD displayed slender perikaryon and shorter synapses. Real-time RT-PCR results showed that the expression levels of NONRATT021972 lncRNA in PC12 cells cultured with OGD were much higher than those in cells cultured in control medium ($p < 0.01$). The experiment was performed in triplicate and repeated three times. The values represent the mean $\pm$ SEM. ** $p < 0.01$ vs. control group

viability of PC12 cells after OGD. The cell viability of PC12 in the control group, OGD group, OGD + NCsiRNA group, OGD + NONRATT021972 lncRNA-siRNA, OGD + BzATP (30 μ M) group, and OGD + BBG (100 nM) group was 1.2 ± 0.09 , 0.70 ± 0.05 , 0.60 ± 0.04 , 1.15 ± 0.09 , 0.59 ± 0.06 , and 1.11 ± 0.09 , respectively ($n = 10$ for each group). There was a significant difference in viability between the OGD group and OGD + NONRATT021972 lncRNA-siRNA group ($p < 0.01$; Fig. 2).

Effects of NONRATT021972 siRNA on the upregulated expression of P2X₇ mRNA in PC12 cells treated with OGD

To study the molecular mechanism for OGD-induced adverse effects on PC12 cells, the expression of P2X₇ mRNA in PC12 cells was measured by real-time PCR. The expression of P2X₇ mRNA in PC12 cells cultured with OGD was 2.3-fold higher than that in cells cultured in control medium ($p < 0.01$; Fig. 3). NONRATT021972 siRNA treatment decreased the P2X₇ mRNA levels by 50 % in PC12 cells treated with OGD. The P2X₇ antagonist, BBG, reduced the expression of P2X₇ mRNA in cells cultured in OGD. There was no significant difference in P2X₇ mRNA levels among the OGD, OGD + scramble siRNA, and OGD + BzATP groups ($p > 0.05$; Fig. 3). Thus, the NONRATT021972 lncRNA siRNA treatment may protect PC12 cells from OGD injury.

Effects of NONRATT021972 siRNA on the upregulated expression of P2X₇ protein in PC12 cells treated with OGD

P2X₇ protein expression was examined by western blotting, and it was normalized to the β -actin internal control. The integrated optical density (IOD) ratio of P2X₇/ β -actin showed that the P2X₇ protein levels in the OGD and OGD + BzATP (30 μ M) groups were significantly increased by 2.3- to 2.4-fold in comparison to that in the control group. After NONRATT021972 siRNA or BBG treatment in the OGD group, the P2X₇ protein level was 50 to 55 % lower than that in the OGD and OGD + BzATP groups ($p < 0.01$; Fig. 4).

Effects of NONRATT021972 siRNA on ATP- or BzATP-induced $[Ca^{2+}]_i$ responses in PC12 cells treated with OGD

To investigate the effects of NONRATT021972 siRNA on OGD-induced Ca^{2+} signals promoted by ATP or BzATP in PC12 cells, NONRATT021972 siRNA was transfected into PC12 cells cultured with OGD. PC12 cells were then cultured in control or OGD medium. Cells cultured in OGD conditions were also treated with scramble siRNA (OGD + NCsiRNA), NONRATT021972 siRNA (OGD + lncsiRNA), P2X₇ agonist (BzATP, 30 μ M; OGD + BzATP), or P2X₇ antagonist (BBG, 100 nM; OGD + BBG). After reperfusion

Fig. 2 The effects of NONRATT021972 siRNA treatment on the viability of PC12 cells subjected to OGD. Cell viability was measured by MTS assays. PC12 cells were seeded in 96-well plates, and they were cultured in control or OGD medium. PC12 cells subjected to OGD were also treated with scramble siRNA (OGD + NCsiRNA), NONRATT021972 siRNA (OGD + lncsiRNA), P2X₇ agonist (BzATP, 30 μ M; OGD + BzATP), or P2X₇ antagonist (BBG, 100 nM; OGD +

BBG) for 48 h. Cells were incubated with MTS for 2 h before measurement of optic density at 490 nm. Inhibition of NONRATT021972 lncRNA by RNA interference improved the cell viability of PC12 cells after OGD. The values represent the mean $\pm$ SEM of ten observations. ** p < 0.01 vs. control group; ## p < 0.01 vs. OGD group

for 48 h, intracellular calcium concentrations ($[Ca^{2+}]_i$) promoted by ATP (100 μ M) or BzATP (30 μ M) were measured simultaneously in each group. The results showed that $[Ca^{2+}]_i$ promoted by ATP (300 nM) or BzATP (30 μ M) in PC12 cells treated with OGD were 1.87- or 1.9-fold higher,

respectively, than those in cells cultured in normal conditions. As described in Fig. 5, $[Ca^{2+}]_i$ levels promoted by ATP (100 μ M) or BzATP (30 μ M) in PC12 cells treated with OGD were inhibited by 38 or 41 %, respectively, after knockdown of NONRATT021972 by transfection of

Fig. 3 The effects of NONRATT021972 siRNA on the expression of P2X₇ mRNA in PC12 cells subjected to OGD. PC12 cells were cultured in control or OGD conditions. PC12 cells subjected to OGD were also treated with scramble siRNA (OGD + NCsiRNA), NONRATT021972 siRNA (OGD + lncsiRNA), P2X₇ agonist (BzATP, 30 μ M; OGD + BzATP), or P2X₇ antagonist (BBG, 100 nM; OGD + BBG) for 48 h.

The expression of P2X₇ mRNA was measured by real-time RT-PCR. Knockdown of NONRATT021972 by siRNA decreased the expression of P2X₇ mRNA in PC12 cells subjected to OGD. The values represent the mean $\pm$ SEM of nine independent experiments. ** p < 0.01 vs. control group; ## p < 0.01 vs. OGD group

Fig. 4 The effects of NONRATT021972 siRNA treatment on the expression of P2X₇ protein in PC12 cells subjected to OGD. The expression of P2X₇ protein was measured by western blotting in PC12 cells cultured in control or OGD conditions. PC12 cells subjected to OGD were also treated with scramble siRNA (OGD + NCsiRNA), NONRATT021972 siRNA (OGD + lncsiRNA), P2X₇ agonist (BzATP, 30 μM; OGD + BzATP), or P2X₇ antagonist (BBG, 100 nM; OGD +

BBG) for 48 h. **a** Representative western blot images. **b** The relative protein level of P2X₇ was quantified by densitometry after normalization to the β-actin levels. NONRATT021972 siRNA treatment markedly inhibited the upregulation of P2X₇ protein expression induced by OGD. The experiments were performed in triplicate and repeated three times. The values represent the mean ± SEM. ***p* < 0.01 vs. control group; ##*p* < 0.01 vs. OGD group

NONRATT021972 siRNA. The P2X₇-specific antagonist, BBG (100 nM), also caused decreases of 40 or 43 % in

[Ca²⁺]_i levels stimulated by ATP (100 μM) or BzATP (30 μM), respectively, in OGD. There was no significant

Fig. 5 The effects of NONRATT021972 siRNA treatment on the [Ca²⁺]_i responses stimulated by P2X₇ agonists (ATP or BzATP) in PC12 cells subjected to OGD. Intracellular calcium concentration ([Ca²⁺]_i) was measured by Fura-2/AM, and the fluorescence ratio (F340/F380) was calculated as an indicator of [Ca²⁺]_i. The intracellular Ca²⁺ induced by ATP (100 μM) or BzATP (30 μM) in PC12 cells subjected to OGD was

increased in comparison with that in the control group. NONRATT021972 siRNA and the P2X₇ antagonist (BBG) markedly inhibited the increasing Ca²⁺ signals stimulated by ATP (100 μM) or BzATP (30 μM) in PC12 cells subjected to OGD. The experiments were performed in triplicate and repeated three times. The values represent the mean ± SEM. **p* < 0.05, ***p* < 0.01 vs. control group; #*p* < 0.05, ##*p* < 0.01 vs. OGD group

difference in $[Ca^{2+}]_i$ levels among the OGD, OGD+ scramble siRNA and OGD+BzATP groups ($p>0.05$; Fig. 5).

Discussion

ATP acting on P2X receptors is involved in signal transmission of autonomic nerves [2, 42–45]. ATP, released during ischemia, can initiate sympathetic neuronal injury and lead to abnormal cardiac function [12–18, 21, 46, 47]. The rat pheochromocytoma PC12 cell line is used as a popular model system to study sympathetic neuronal function [24]. Our experiments showed that the expression levels of NONRATT021972 lncRNA in PC12 cells subjected to OGD were significantly higher than those in cells cultured in control medium. LncRNAs have important effects on both normal development and diseases of the nervous system [28, 32, 48]. Thus, the NONRATT021972 lncRNA may relate to the pathophysiological changes of PC12 neuronal injury induced by OGD. Our results showed that the viability of PC12 cells was decreased under OGD conditions and that the decreased viability was increased after pretreatment with NONRATT021972 lncRNA siRNA. These results suggested that NONRATT021972 lncRNA siRNA treatment can reverse the viability effects of OGD in PC12 cells.

Previous data in our laboratory showed that the P2X₇ receptor is implicated in the pathological injury of sympathetic nerves [17, 18, 21, 24, 34, 35]. The P2X₇ receptor plays important roles in the pathological process of inflammation or ischemic injury [2, 9, 21, 27, 42, 45], and purinergic signaling participates in the regulation of sympathetic function [43, 46]. P2X receptors can be activated by ATP, and ATP released from damaged cells may upregulate and activate the P2X₇ receptor and initiate neuronal injury [2, 24, 27, 49, 50]. The upregulated expression of P2X₇ mRNA and protein in PC12 cells is related to OGD [34]. Our studies showed that the expression levels of P2X₇ mRNA and protein were significantly increased after PC12 cells subjected to OGD were exposed to a selective P2X₇ agonist (BzATP). The overexpression levels of P2X₇ mRNA and protein were decreased in PC12 cells pretreated with NONRATT021972 siRNA in our study. Therefore, treatment with NONRATT021972 siRNA in PC12 cells may reduce P2X₇ expression and prevent the sympathetic neuronal damage in OGD pathological conditions.

The accumulation of ATP and overactivation of the P2X₇ receptor can cause neuronal injury by calcium (Ca^{2+}) overload [9, 27, 45]. Our results showed that the Ca^{2+} signals induced by P2X₇ agonists (ATP or BzATP) in the OGD group were increased. After PC12 cells subjected to OGD were cotreated with NONRATT021972 siRNA, the Ca^{2+} signals induced by ATP or BzATP stimuli were significantly

decreased in comparison to the signals in the OGD-alone group. These results indicated that NONRATT021972 siRNA treatment inhibits the $[Ca^{2+}]_i$ concentration in PC12 cells after OGD. Our data showed that treatment with NONRATT021972 siRNA may decrease the overactivation of the P2X₇ receptor and attenuate the sympathetic neuronal injury after exposure to OGD. It is possible that the NONRATT021972 lncRNA may be involved in the abnormal changes of cell viability and $[Ca^{2+}]_i$ peak mediated by the P2X₇ receptor in PC12 cells after exposure to OGD. Silencing NONRATT021972 can relieve the sympathetic neuronal injury after exposure to OGD.

In summary, our results showed that treatment with NONRATT021972 siRNA may inhibit the upregulation of the P2X₇ receptor induced by OGD in PC12 cells and may reduce the P2X₇ receptor-mediated neuronal injury induced by OGD. The NONRATT021972 lncRNA may participate in the pathophysiological process of PC12 neuronal injury induced by OGD. Treatment with NONRATT021972 siRNA improved the pathological changes of the cell viability and $[Ca^{2+}]_i$ peaks affected by OGD and prevented the sympathetic neuronal injury following ischemia.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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