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Knockdown of lncRNA NKILA suppresses sevoflurane-induced neuronal cell injury partially by targeting miR-205-5p/ELAVL1 axis

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Abstract. Sevoflurane (Sev) is a wildly used volatile anesthetic agent that induces neurotoxicity. Long non-coding RNAs (lncRNAs) have been demonstrated to be involved in Sev-induced neuronal injury. Here, we investigated the role of NF-kappaB-interacting lncRNA (NKILA) in Sev-treated human cortical neurons (HCN). From RT-qPCR, Sev dose-dependently increased HCN NKILA transcript expression. Neurotoxicity of Sev was detected using MTT, flow cytometry, Western blotting, and inflammatory mediator assays. Consequently, Sev reduced HCN viability and levels of Bcl-2, SOD, and GSH in HCN, and promoted HCN apoptosis rate and levels of cleaved-caspase-3, Bax, MDA, TNF-α, IL-6, and IL-1β. Silencing NKILA suppressed Sev-induced above effects. DIANA and starbase databases predicted the potential target relationship between miR-205-5p and NKILA or embryonic lethal abnormal vision-like 1 (ELAVL1); dual-luciferase and RIP confirmed these interactions. NKILA could increase ELAVL1 expression by regulating miR-205-5p. miR-205-5p overexpression and ELAVL1 knockdown could mimic effects of NKILA silencing in Sev-induced HCN. Deleting miR-205-5p and restoring ELAVL1 respectively abolished the neuroprotective effect of NKILA knockdown and miR-205-5p upregulation under Sev anesthesia. In conclusion, Sev induced neuronal cell apoptosis, inflammatory response and oxidative stress through NKILA/miR-205-5p/ELAVL1 axis and caspase-3 and Bax/Bcl-2 pathway. Inhibiting NKILA might be a potential therapeutic strategy for Sev neurotoxicity.

Key words: Sevoflurane — Neuron — NKILA — miR-205-5p — ELAVL1

Introduction

Sevoflurane (Sev), fluorinated methyl isopropyl ether, is a volatile anesthesia and is inspired through the nasal cavity (Gao et al. 2020). Sev is commonly used as a general anesthetic in patients ranging from neonates to the aged (Lu et al. 2020). However, surgical general anesthesia is always coupled with inevitable brain injury and neurological deficits (Sun 2010). Clinically, general anesthesia is found to be associated with postoperative cognitive dysfunction and neurodegeneration disorder in aged patients (Cascella and Bimonte 2017), and with neurodevelopmental impairment and behavioral dysfunction in pediatric patients (Walters and Paule 2017). Moreover, accumulating *in vivo* and *in vitro* experiments show that Sev could induce neurotoxicity (Liu M et al. 2020; Liu T et al. 2020). Accordingly, continuous exploration the precise mechanism of Sev-induced human neuronal cell injury is vitally important at present.

Several years ago, a microarray study revealed that long non-coding RNAs (lncRNAs) together with their messenger RNAs (mRNAs) targets were abnormally expressed in Sev-induced hippocampal neuronal apoptosis (Chen et al. 2016). Since then, many studies have shown that lncRNAs are involved in Sev-induced neurontoxicity *via* serving as naturally competing endogenous RNAs (ceRNAs), such as Rik-203/microRNA (miRNA)-466l-3p/brain-derived neurotrophic factor (Zhang et al. 2020a). LncRNAs are a group of >200 bp RNAs and show specific spatiotemporal expression profiles. Nuclear factor-kappaB (NF-κB)-interacting

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lncRNA (NKILA) is closely associated with NF-κB/p65 signaling pathway, a pro-inflammatory signaling cascade and a potential therapeutic target in postoperative cognitive dysfunction after Sev anesthesia (Zheng et al. 2017). NKILA is a killer regulator that suppresses the growth and deterioration of human cancers (Bird 2018; Tian et al. 2020). In neurological diseases, NKILA has been implicated in neuronal cell injuries by regulating cell viability, apoptosis, autophagy and necrosis (Jia et al. 2018; Wang M et al. 2018). However, it is unclear that whether NKILA participates in Sev-induced neurotoxicity.

Embryonic lethal abnormal vision-like 1 (ELAVL1; also known as HuA and HuR) is recognized as a therapeutic target in various human diseases (Vosgha et al. 2014; Schultz et al. 2020). Essentially, ELAVL1 is a ubiquitously expressed RNA-binding protein, and the interplay between ELAVL1 and miRs could determine cellular stress response to some extent (Meisner and Filipowicz 2010; Chang and Hla 2014). miRNA (miR)-205-5p and oxidative/inflammatory stress have reciprocal regulatory responses (Magenta et al. 2016; Yeh et al. 2016), and *vice versa* (Yeh et al. 2016; Oltra et al. 2020). However, the cross-talk between ELAVL1 and miR-205-5p is unknown.

Herein, the role of NKILA in Sev-induced neurotoxicity in human cortical neurons (HCN) was examined by monitoring cell viability, apoptosis, inflammatory and oxidative responses. Moreover, the potential ceRNA pathway involving NKILA, miR-205-5p and ELAVL1 was further explored.

Materials and Methods

Cell culture and cell transfection

HCN cell line HCN-2 (CRL-10742; ATCC, Manassas, VA, USA) was cultured in DMEM (M22650; R&D SYSTEM, Minneapolis, MN, USA) containing 10% fetal bovine serum (FBS; S12550H; R&D SYSTEM). HCN-2 cells were incubated in a humidified atmosphere containing 5% CO_2 at 37°C, and employed in this study within passage 3–10 since the cell line was firstly resuscitated.

Small interfering RNA (siRNA) targeting NKILA (si-NKILA) and si-ELAVL1 were designed and synthesized to silence NKILA and ELAVL1, respectively. miR-205-5p mimic/inhibitor (miR-205-5p/anti-miR-205-5p) were designed to overexpress and knockdown miR-205-5p, respectively. For overexpression of ELAVL1, pCS2+vector (miaolingbio, Wuhan, China) was used. Similarly, corresponding negative controls (NC) included non-targeting siRNA (si-NC), miR-NC mimic/inhibitor (miR-NC/anti-miR-NC), and empty pCS2+vector (vector). HCN-2 cells were transfected with above oligos and vectors using NeuroPORTER Transfection Reagent Kit (Genlantis, San Diego, CA, USA) according to manufacturer's recommendation. The sequence of above oligos was si-NKILA, 5'-UUCAGGAUGAAUUCGCUCUGGdTdT-3'; si-ELAVL1, 5'-GGUUUAUGACCAUUGAAACUGdTdT-3'; miR-205-5p, 5'-UCCUUCAUUCCACCGGAGUCUG-3'; anti-miR-205-5p, 5'-CAGACTCCGGTGGAATGAAGGA-3'; si-NC, 5'-GCUACGAUCUGCCCAAGAUdTdT-3'; miR-NC, 5'-UUUGUACUACACAAAAGUACUG-3'; anti-miR-NC, 5'-UCUACUCUUUCUAGGAGGUUGGA-3'.

Sev treatment

Sev machine (Sevorane, Abbott Laboratories Queenborough, UK), Sev vaporizer (Drage, Lubeck, Germany) and a sealed glass chamber for cell culture were connected in sequence. For Sev treatment, HCN-2 cells were transferred in humidified atmospheres containing different concentrations of Sev (1%, 2% and 4%), 5% CO_2, 21% O_2, and balanced with N_2. After Sev treatment for 6 h, HCN-2 cells were harvested for further assays. HCN-2 cells without Sev treatment served as Control group.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability of HCN-2 cells was detected using MTT Cell Viability & Proliferation Assay (ScienCell, San Diego, CA, USA) according to standard method. Briefly, HCN-2 cells in Control and Sev groups were incubated with pale yellow tetrazolium MTT (5 mg/ml), which was converted to purple formazan crystals. Spectrophotometric determination of soluble crystals in dimethylsulfoxide (DMSO) was performed on a microplate reader (Applied Biosystems, Carlsbad, CA, USA) at 570 nm wavelength.

RT-qPCR

Total RNA was prepared by TRIzol Plus RNA Isolation Reagents (Invitrogen, Carlsbad, CA, USA). The RNA was analyzed using 5× All-In-One RT MasterMix (Applied Biological Material, Richmond, BC, Canada) and BlasTaq™ 2× qPCR MasterMix (Applied Biological Material). Housekeeping genes actin and U6 were internal controls for expression of NKILA, ELAVL1 and miR-205-5p. RNA expression levels were overall evaluated by comparative Cycle Threshold (Ct) values using $2^{-\Delta\Delta Ct}$ formula. For RT-qPCR analysis, primers were shown in Table 1.

Flow cytometry

After Sev treatment for 6 h, HCN-2 cells (2.5×10^5 cells) were collected and then labelled with Annexin V-Fluorescein

Isothiocyanate (FITC)/Propidium Iodide (PI) Apoptosis Detection Kit (Vazyme, Nanjing, China). In brief, cells were harvested using tyrosine (without EDTA), re-suspended in the 1× Binding Buffer, and incubated with the Annexin V-FITC and PI staining solution according to the manufacturer's instruction. FACScan flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) equipped with FlowJo v10.0.7 software (Tree Star, San Carlos, CA, USA) was used to analyze labelled cells.

Western blotting

Protein expression in cell lysate of HCN-2 cells was examined, and total proteins were prepared by RIPA lysis buffer (Beyotime). Western blotting was carried out using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and antibody incubation. Next, horseradish peroxidase (HRP) signal was detected by BeyoECL Star kit (Beyotime). The antibodies (Abcam, Cambridge, UK) used included Bcl-2 (ab32124, 1:1,000), Bax (ab32503, 1:5,000), cleaved-caspase-3 (ab2302, 1:250), GAPDH (ab9485, 1:2,500), ELAVL1 (ab200342, 1:1,000), and secondary antibody (ab205718, 1:50,000). Finally, quantification of blots was carried out on Image-ProPlus software.

Assay kits and enzyme-linked immunosorbent assay (ELISA)

The levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) in cell lysate of HCN-2 cells were respectively detected *via* Lipid Peroxidation MDA Assay Kit (Beyotime, Shanghai, China), Total Glutathione Assay Kit (Beyotime) and Total SOD Assay Kit with WST-8 (Beyotime). IL-6, TNF-α, and IL-1β in the cell supernatant were severally determined using Human IL-6 ELISA Kit (Beyotime), Human TNF-α ELISA Kit (Beyotime), and Human IL-1α ELISA Kit (Beyotime).

Table 1. The sequences of primers for RT-qPCR used in this study

	Sequences (5'–3')
NKILA	F: AAGCTCACTCTTGGAGCCAC
	R: GGGGTAGACGCTGGGTATTG
ELAVL1	F: TGGTAGTTAGACCCCAGGCA
	R: TGCGGCTTGGCAAATTACAC
miR-205-5p	F: GGTATGATCCTTCATTCCACCGG
	R: CTCAACTGGTGTCGTGGAGTC
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: CAAATTCCATGGCACCGTCA
	R: GACTCCACGACGTACTCAGC

F, forward; R, reverse.

Dual-luciferase reporter assay

Binding site of miR-205-5p in NKILA and ELAVL1 was predicted using LncBase Predicted v.3 and starbase databases. Predicted complementary sites in NKILA and ELAVL1 3'UTR were mutated from …GAUUGCGAGGAAUGAAGG… to …UCUGGUAAUAGCCUCGUU… and from …GAGUGAAGG… to …UCGGUGCUA…, respectively. Then, the wild type (WT) and mutant (MUT) NKILA and ELAVL1 3'UTR were inserted into the pMIR-REPORT vector (Promega, Madison, WI, USA), and were transfected into HCN-2 cells together with miR-205-5p or miR-NC using NeuroPORTER Transfection Reagent Kit (Genlantis), followed by Dual-Luciferase Reporter assay system (Promega) analysis

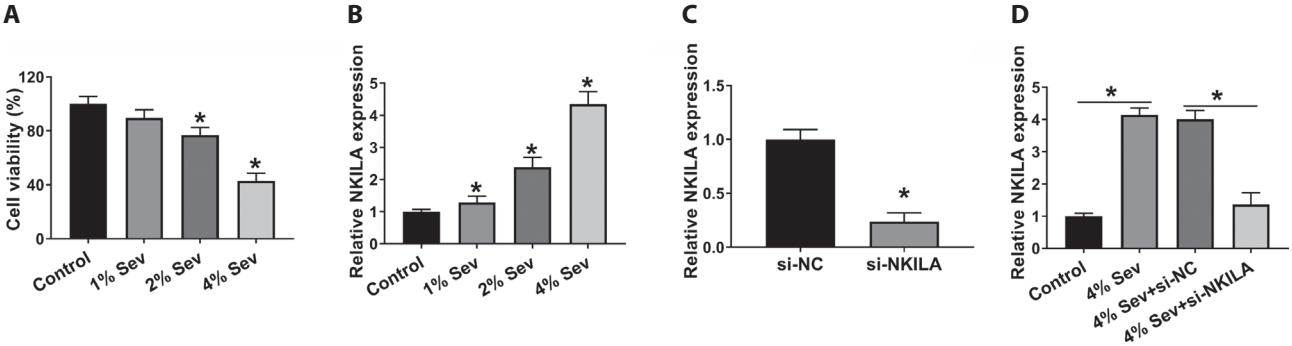

Figure 1. Sev reduced neuronal cell viability and induced NKILA expression. In HCN-2 cells both in Control group (without Sev treatment) and Sev groups (with 1%, 2% or 4% Sev treatment), MTT assay examined cell viability (**A**), RT-qPCR detected expression of NKILA (**B**). RT-qPCR detected expression of NKILA in HCN-2 cells transfected with si-NC or si-NKILA (**C**), and HCN-2 cells both in Control group and 4% Sev groups with pre-transfection of si-NKILA or si-NC (**D**). * $p < 0.05$. HCN, human cortical neurons; NKILA, NF-kappaB-interacting lncRNA; Sev, sevoflurane.

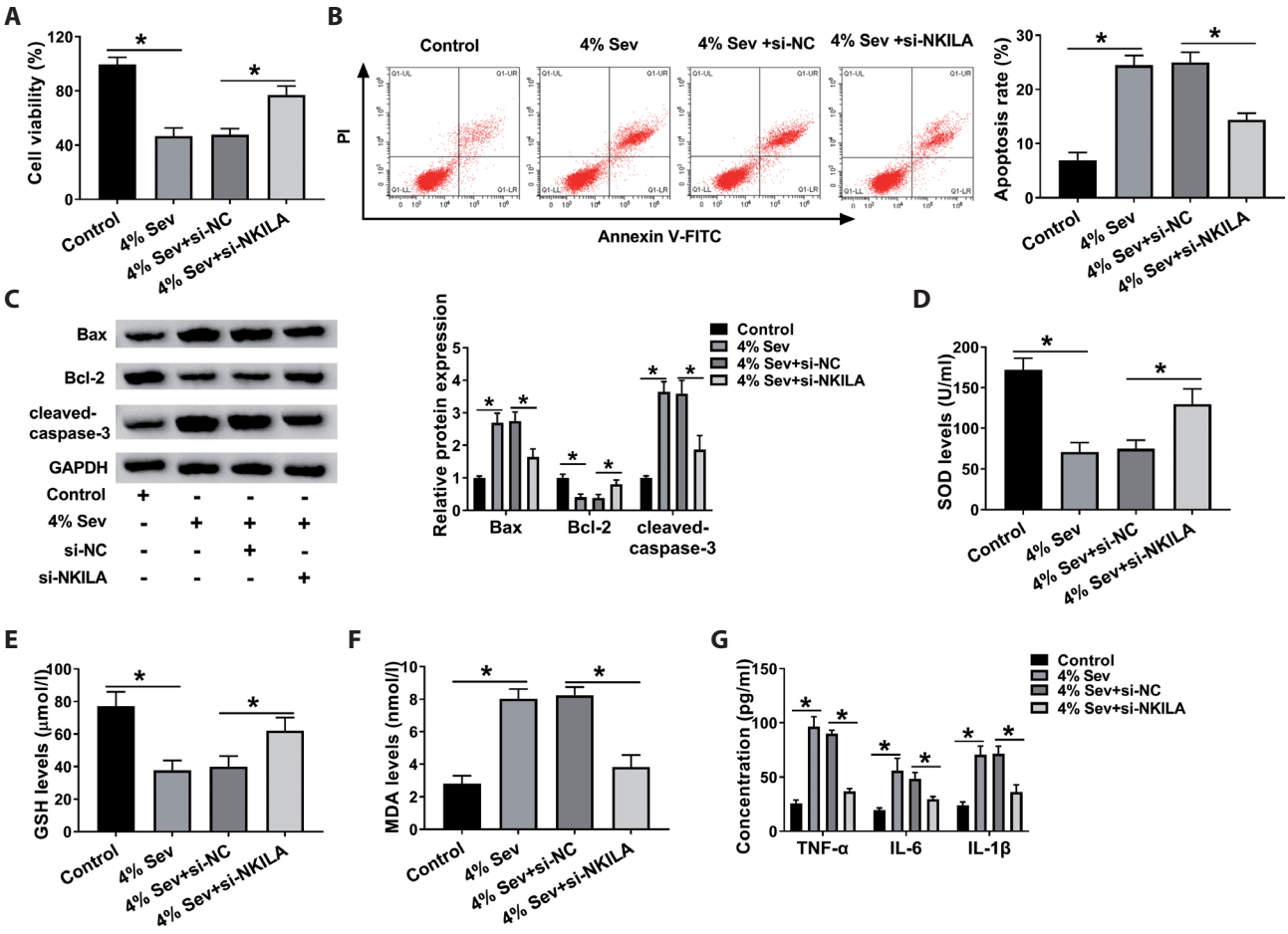

Figure 2. Silencing NKILA alleviated Sev-induced neuronal cell injury *in vitro*. HCN-2 cells in 4% Sev group were pre-transfected with si-NKILA or si-NC. **A.** MTT assay examined cell viability (%). **B.** Flow cytometry measured apoptosis rate (%). **C.** Western blotting detected protein expression of Bcl-2, Bax and cleaved-caspase-3, normalized to GAPDH expression. Total SOD (**D**), GSH (**E**) and MDA (**F**) levels in the cell lysate were measured. **G.** ELISA kits determined the levels of TNF-α, IL-6, and IL-1β in the cell supernatant. * $p < 0.05$. For abbreviations, see Figure 1.

RNA immunoprecipitation

Cell lysate of HCN-2 cells was processed using Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA). Protein A/G magnetic beads were coated with anti-Ago2 (10686-1-AP, 1:100; Proteintech, Wuhan, China) or anti-IgG (10283-1-AP, 1:100; Proteintech) at 4°C overnight, and the immunoprecipitated RNA-protein complex was incubated with the proteinase K to obtain immunoprecipitated RNA samples. Lastly, the RNA expression of NKILA and miR-205-5p was analyzed by RT-qPCR.

Statistical analysis

Experimental data involved in this article are presented as mean ± standard deviation and performed on GraphPad Prism V5.0 software. The statistical difference was determined by one-way analysis of variance or Student's *t* test, and statistical significance level was set at $p < 0.05$.

Results

Sev induced NKILA high expression and silencing NKILA alleviated Sev-induced neuronal cell injury in vitro

Sev treatment reduced cell viability of HCN-2 cells and up-regulated NKILA expression in a concentration-dependent manner (Fig. 1A,B). The si-NKILA transfection knocked down NKILA transcript levels in HCN-2 cells and in 4% Sev-treated HCN-2 cells (Fig. 1C,D). Sev decreased HCN-2 cell viability which was blunted by NKILA silencing (Fig. 2A). FCM analysis showed that apoptosis rate of HCN-2

cells was promoted in response to 4% Sev treatment, and this promotion was blocked by decreasing NKILA transcript levels following siRNA transfection (Fig. 2B). Molecularly, the anti-apoptosis effect of NKILA depletion was accompanied with increased Bcl-2 expression and decreased Bax and cleaved-caspase-3 expression (Fig. 2C). Additionally, 4% Sev induced oxidative stress and inflammatory response in HCN-2 cells, as evidenced by the depression of total SOD and GSH levels (Fig. 2D,E), and the enhancement of MDA, TNF-α, IL-6, and IL-1β levels (Fig. 2F,G). These results demonstrated an upregulation of NKILA transcripts in human neurons under Sev treatments and the ability of NKILA silencing to block Sev-induced apoptosis, inflammation and oxidative stress.

NKILA was a ceRNA for miR-205-5p in regulating ELAVL1 in HCN

NKILA was predicted to bind miR-205-5p according to LncBase Predicted v.3 database in DIANA (Fig. 3A), and overexpression of miR-205-5p *via* its mimic transfection significantly reduced the luciferase activity of reporter vectors carrying NKILA WT (Fig. 3B,C). In addition, the luciferase activity of NKILA MUT vectors was unaffected by miR-205-5p mimic (Fig. 3C). Notably, NKILA and miR-205-5p were concurrently enriched by Ago2 in cell lysate of HCN-2 cells (Fig. 3D). Thus, we considered that miR-205-5p was sponged by NKILA in HCN. Expression of miR-205-5p was upregulated by NKILA knockdown and downregulated by 4% Sev treatment in HCN-2 cells (Fig. 3E,F).

Downstream target gene for miR-205-5p was further identified, and starbase website suggested a potential binding site between miR-205-5p and ELAVL1 3'UTR WT (Fig. 4A). Furthermore, dual-luciferase reporter assay showed the decline of luciferase activity of ELAVL1 3'UTR WT vectors instead of the MUT vectors in miR-205-5p-overexpressed HCN-2 cells (Fig. 4B). These data validated the direct relationship between ELAVL1 and miR-205-5p in HCN. Expression of ELAVL1 both at mRNA level and protein level was downregulated by miR-205-5p overexpression and upregulated by 4% Sev stimulation in HCN-2 cells (Fig. 4C–F).

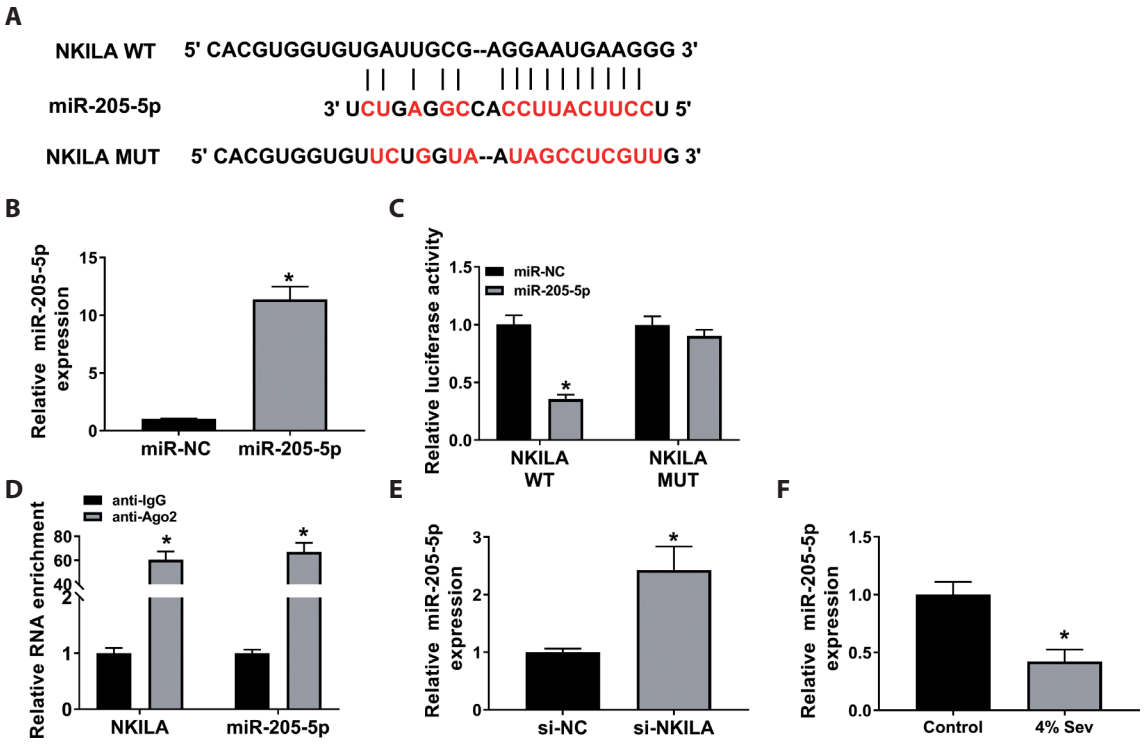

Figure 3. miR-205-5p was targeted by NKILA. **A.** LncBase Predicted v.3 database indicated a binding sequence between NKILA WT and miR-205-5p, and then NKILA MUT was generated by mutating these sites. **B.** RT-qPCR detected miR-205-5p expression in HCN-2 cells transfected with designed miR-205-5p or miR-NC. **C.** Dual-luciferase reporter assay tested the luciferase activities of reporter NKILA WT or MUT vectors in HCN-2 cells transfected with miR-205-5p or miR-NC. **D.** RNA immunoprecipitation assay identified the enrichment of NKILA and miR-205-5p in cell lysate of HCN-2 cells. RT-qPCR detected miR-205-5p expression in HCN-2 cells in si-NKILA and si-NC transfection groups (**E**) and in Control and 4% Sev groups (**F**). * $p < 0.05$. WT, wild type; MUT, mutant. For more abbreviations, see Figure 1.

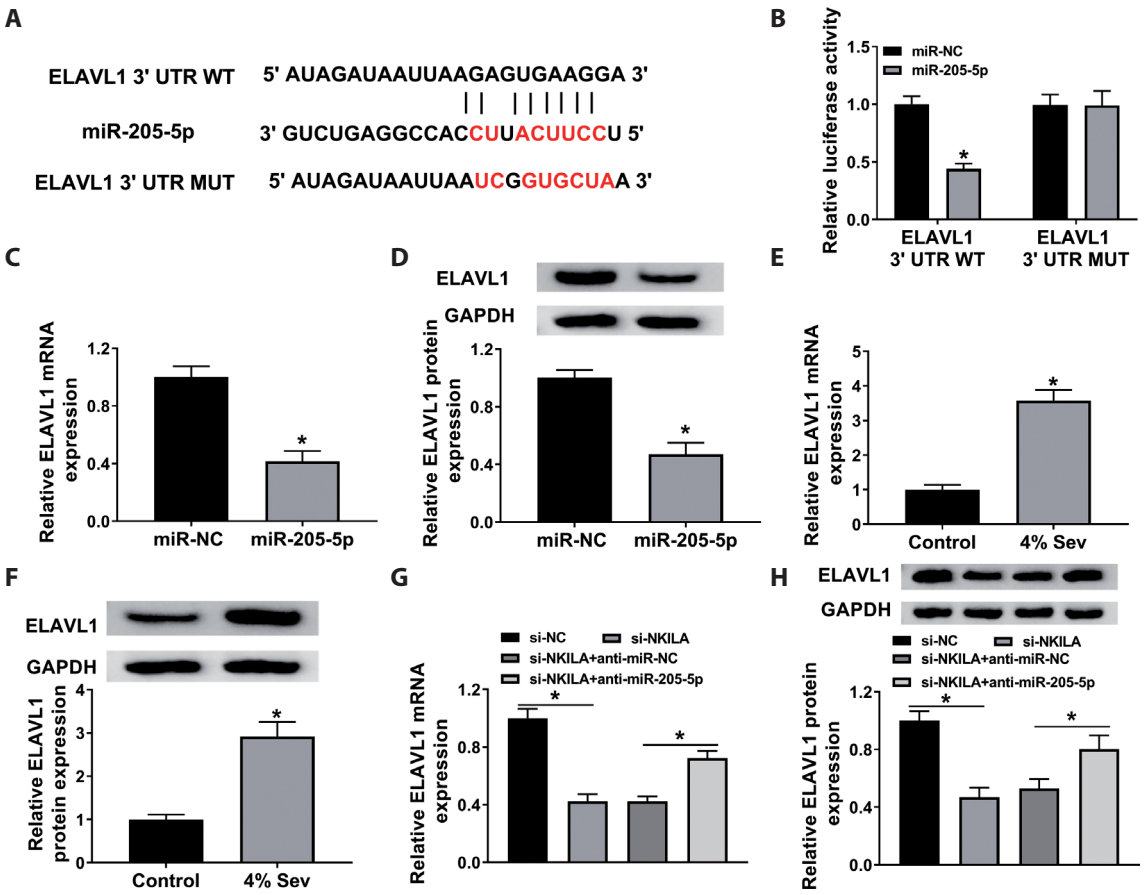

Figure 4. ELAVL1 was a target gene for miR-205-5p. **A.** ELAVL1 3'UTR MUT was constructed by mutating the predicted miR-205-5p-binding sites in ELAVL1 3'UTR WT according to starbase website. **B.** Dual-luciferase reporter assay tested the luciferase activities of ELAVL1 3'UTR WT and MUT vectors in HCN-2 cells with co-transfection of miR-205-5p or miR-NC. RT-qPCR and Western blotting detected ELAVL1 mRNA expression and protein expression in HCN-2 cells transfected with miR-205-5p or miR-NC (**C, D**), HCN-2 cells in Control and 4% Sev groups (**E, F**), and HCN-2 cells transfected with si-NC alone and si-NKILA alone or together with anti-miR-205-5p or anti-miR-NC (**G, H**). * $p < 0.05$. For abbreviations, see Figure 3.

Strikingly, si-NKILA-mediated NKILA knockdown resulted in low expression of ELAVL1, and this downregulation could be salvaged in the co-presence of anti-miR-205-5p (Fig. 4G,H). This data suggested that ELAVL1 expression was modulated by NKILA *via* miR-205-5p.

NKILA/miR-205-5p/NKILA axis regulated Sev-induced neuronal cell injury in vitro

Anti-miR-205-5p transfection was performed to knock down miR-205-5p expression in HCN-2 cells and 4% Sev-induced HCN-2 cells with NKILA silencing (Fig. 5A,B). Exhausting NKILA improved cell viability of HCN-2 cells under 4% Sev treatment, and this improvement was abrogated by exhausting miR-205-5p (Fig. 5C). Cell apoptosis in NKILA-downregulated HCN-2 cells under 4% Sev treatment was aggravated by simultaneously depressing miR-205-5p, as indicated by higher apoptosis rate, diminished Bcl-2 expression and elevated Bax and cleaved-caspase-3 expression (Fig. 5D,E). Blocking NKILA rescued total SOD and GSH levels whereas diminished MDA, TNF-α, IL-6, and IL-1β levels in 4% Sev-treated HCN-2 cells, and these effects were overall counteracted by additionally blocking miR-205-5p (Fig. 5F–I). These outcomes showed that miR-205-5p downregulation could abrogate the effects of NKILA silencing in Sev-induced HCN injury. However, overexpressing miR-205-5p could suppress 4% Sev-induced cell viability inhibition, apoptosis, and inflammatory and oxidative responses (Fig. 6C–I); conversely, restoring ELAVL1 *via* vector transfection significantly abated the protective effects of miR-205-5p overexpression in 4% Sev-induced HCN-2 cells (Fig. 6A–I). These results demonstrated that high ELAVL1 abrogated the protective role of miR-205-5p in HCN under Sev treatment.

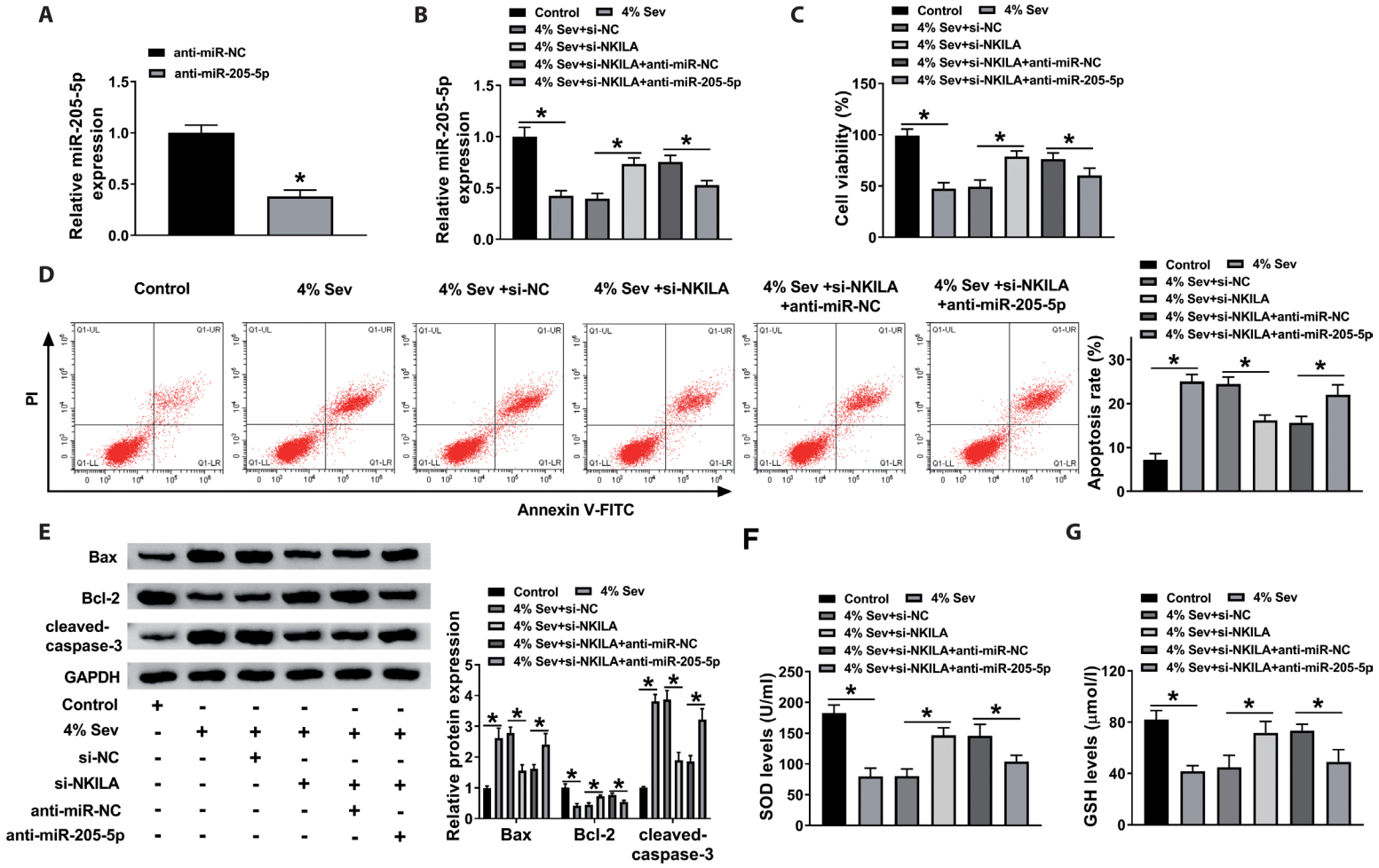

Figure 5. Deleting miR-205-5p abolished the suppressive role of NKILA exhaustion in Sev-induced neuronal cell injury *in vitro*. **A.** RT-qPCR detected miR-205-5p expression in HCN-2 cells transfected with anti-miR-205-5p or anti-miR-NC. HCN-2 cells in 4% Sev group were pre-transfected with si-NC alone and si-NKILA alone or along with anti-miR-205-5p or anti-miR-NC. **B.** RT-qPCR detected miR-205-5p expression. **C.** MTT assay examined cell viability (%). **D.** Flow cytometry measured apoptosis rate (%). **E.** Western blotting detected protein expression of Bcl-2, Bax and cleaved-caspase-3, normalized to GAPDH expression. Total SOD (**F**), GSH (**G**) and MDA (**H**) levels in the cell lysate were measured after transfection paradigms. **I.** ELISA kits determined the levels of TNF-α, IL-6, and IL-1β in the cell supernatant. * $p < 0.05$. For abbreviations, see Figure 1.

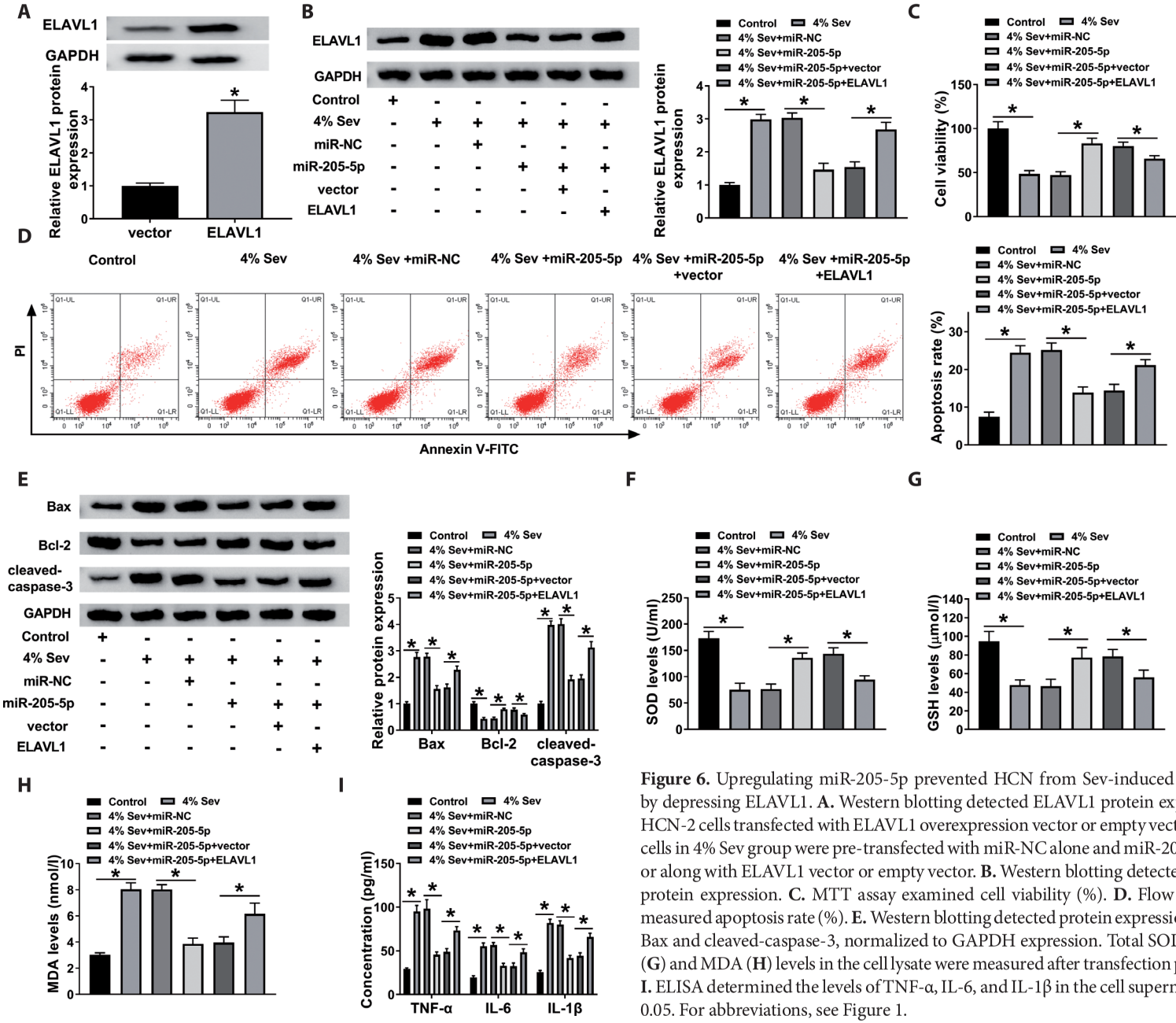

Figure 6. Upregulating miR-205-5p prevented HCN from Sev-induced cell injury by depressing ELAVL1. **A.** Western blotting detected ELAVL1 protein expression in HCN-2 cells transfected with ELAVL1 overexpression vector or empty vector. HCN-2 cells in 4% Sev group were pre-transfected with miR-NC alone and miR-205-5p alone or along with ELAVL1 vector or empty vector. **B.** Western blotting detected ELAVL1 protein expression. **C.** MTT assay examined cell viability (%). **D.** Flow cytometry measured apoptosis rate (%). **E.** Western blotting detected protein expression of Bcl-2, Bax and cleaved-caspase-3, normalized to GAPDH expression. Total SOD (**F**), GSH (**G**) and MDA (**H**) levels in the cell lysate were measured after transfection paradigms. **I.** ELISA determined the levels of TNF-α, IL-6, and IL-1β in the cell supernatant. * $p < 0.05$. For abbreviations, see Figure 1.

Discussion

With its analgesic and anesthetic effect, Sev also has roles in neuronal injury and oncogenesis (Lu et al. 2020; Xu Y et al. 2020). In Sev promotion or suppression of neuronal dysfunction, non-coding RNAs, including lncRNAs and miRNAs are differently expressed, and the dysregulation of these RNAs can alter downstream functional genes in neural progenitor cells (Zhang et al. 2019, 2020a) and hippocampal neurons (Chen et al. 2016; Wang JY et al. 2018; Xu W et al. 2020). In addition, Sev impaired HCN cell proliferation partially through post-transcriptional suppression of CCNA2 *via* miR-19-3p (Zhao et al. 2018), and HCN cell apoptosis was increased at least by activating miR-325-3p-targeted Nupr1 (Xu L et al. 2020). However, whether there was lncRNA/miRNA/mRNA ceRNA regulatory network was unclear in Sev-damaged HCN. Conversely, Lin et al. (2011) stated that Sev (6%) at high concentration did not cause cell plasma membrane injury to human neuron-like cells (differentiated SH-SY5Y), as assessed by lactate dehydrogenase (LDH) release.

In this study, our data showed that neuronal toxicity was induced by 2–4% Sev in HCN-2 cells, which was accompanied by NKILA upregulation in a Sev concentration-dependent manner. Functionally, silencing NKILA could improve cell viability and suppress HCN Sev-induced apoptosis, oxidative stress and inflammatory factors. Our work unveils a lncRNA program in Sev-induced HCN injury. Notably, a NKILA/miR-205-5p/ELAVL1 axis was shown to potentially underlie the Sev-induced apoptosis, inflammation and oxidative stress in HCN cells. Conversely, the apoptotic caspase-3 and Bax/Bcl-2 pathway was inactivated by NKILA knockdown in HCN under Sev treatment.

miR-205-5p is highly conserved and plays important roles in both normal physiology and tumorigenesis (Vosgha et al. 2014; Chen S et al. 2018), as well as neurotoxicity (Rivera-Aponte et al. 2020; Zhang et al. 2020b). Expression of miR-205-5p was downregulated in neuronal damages, such as Parkinson's disease-related brains and MPP^+-induced dopaminergic neurons (Cho et al. 2013; Chen Q et al. 2018; Zhang et al. 2020b), high glucose-stimulated astrocytes (Rivera-Aponte et al. 2020), and Sev-anesthetized HCN (this study). On one hand, downregulation of miR-205-5p was conductive to apoptosis, inflammation and oxidative stress in Sev-treated HCN with NKILA knockdown; on the other hand, restoring miR-205-5p could mimic the role of NKILA silencing in Sev-exposed HCN to mitigate Sev-mediated inflammatory and oxidative injuries. This work demonstrated a protective role of miR-205-5p in Sev neurotoxicity.

ELAVL1 is a membrane of the ELAV family (HuR/ELAVL1, HuB/ELAVL2, HuC/ELAVL3, and HuD/ELAVL4) (Good 1997). Here, a target binding between miR-205-5p and ELAVL1 3'UTR in HCN was observed, and ELAVL1 expression was lower by miR-205-5p mimic or NKILA silencing. Additionally, increased ELAVL1 levels could counteract or attenuate miR-205-5p-mediated HCN neuroprotection under Sev treatments. These outcomes shed light on a direct regulation of miR-205-5p on ELAVL1, suggesting that miR-205-5p is a novel ELAVL1 regulator. Functionally, miR-205-5p might weaken Sev-induced oxidative stress, apoptosis, and inflammation by interacting with ELAVL1. We observed that high ELAVL1 might be responsible to Sev-induced inflammatory and oxidative stresses, and low ELAVL1 mimicked the repression of NKILA silencing and miR-205-5p overexpression in Sev-induced HCN *in vitro*. However, previous evidence showed that ELAVL1 loss contributed to glutamate-induced oxidative damage and necrotic response in hippocampal cells (Skliris et al. 2015). This diversity in ELAVL1 function in neurotoxicity might reflect differences in neurotoxic inducers. Normally, ELAVL1 displays nuclear localization, and it is redistributed to the cytoplasm during senescence or under stress conditions (Kotta-Loizou et al. 2014; Zucal et al. 2015). Whereas we examined ELAVL1 actions under Sev neurotoxicity, we did not examine its potential changes in subcellular distribution.

There are several signaling pathways that have been shown to participate in Sev-induced neurontoxicity, such as AMPK/SIRT1 and PI3K/AKT (Yang et al. 2020), PTEN/Akt/mTOR (Li et al. 2017), MEK/ERK (Peng et al. 2018), and NF-κB (Liu M et al. 2020). These signaling pathways were not further examined in this study. In sum, we demonstrated that Sev-induced NKILA upregulation and sequestration of miR-205-5p in HCN-2 cells resulted in ELAVL1-mediated cell apoptosis, inflammation and oxidative stress *in vitro*. NKILA knockdown might protect HCN from Sev anesthesia-evoked injury through NKILA/miR-205-5p/ELAVL1 ceRNA axis and caspase-3 and Bax/Bcl-2 pathway. Therefore, we suggested that NKILA may be a potential therapeutic target to avoid the detrimental effect on human neurons during Sev anesthesia.

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

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