ORIGINAL RESEARCH

SESN1, as a potential target for postoperative cognitive dysfunction, attenuates sevoflurane-induced neuronal cell damage in the hippocampus
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Abstract
Postoperative cognitive dysfunction (POCD) is a devastating complication with long-term consequences, and new therapeutic targets and drugs are still needed for the treatment of POCD. Sestrin are a family of stress-inducing proteins that regulate cellular metabolic networks. However, the possible effects of Sestrin on POCD were still unclear. This study aimed to investigate the effects of SESN1 in postoperative cognitive dysfunction (POCD) cell model and reveal its mechanism. We constructed an in vitro model of POCD by treating primary rat hippocampal neurons with sevoflurane. Herein, we noticed SESN1 enhanced cell viability induced by sevoflurane. Further, SESN1 improved sevoflurane-induced cell inflammation. We further found that SESN1 improved sevoflurane induced reactive oxygen species (ROS) production and inhibited apoptosis. Mechanically, SESN1 restrained NOD-like receptor thermal protein domain 3 (NLRP3) inflammasome activation and therefore suppressed POCD. In conclusion, SESN1, as a potential target for postoperative cognitive dysfunction, attenuates sevoflurane-induced neuronal cell damage in the hippocampus. These findings will provide guidance for the mechanism study of POCD and future drug development for treatment of POCD.

Keywords
Postoperative cognitive dysfunction (POCD); SESN1; Inflammation; Sevoflurane; NLRP3 inflammasome

1. Introduction
Postoperative cognitive dysfunction (POCD) is a devastating complication with long-term consequences, defined as impaired memory, attention and information processing that occurs after anesthesia [1]. Studies have shown that approximately 10% of surgical patients develop POCD [2, 3]. Compared to patients without POCD, patients with POCD had significantly higher mortality rate, and higher dependence on social security [4]. Despite extensive research efforts in recent decades, the pathogenesis of POCD remains unknown. Neuroinflammation and oxidative stress in the brain are known to be critical in the development of POCD [5, 6]. While inflammation or oxidative stress caused by surgery can lead to neuroinflammation and brain damage, and eventually cognitive impairment, recent reports suggest that anesthetics used during surgery can also trigger cognitive impairment [7, 8]. New therapeutic targets and drugs are still needed for the treatment of POCD.

Sestrin are a family of stress-inducing proteins that regulate cellular metabolic networks [9]. SESN1 inhibits NLRP3 inflammasome activation and foam cell formation [10]. Overexpression of SESN1 reduces the expression of pro-inflammatory cytokines in macrophages and throughout aortic tissue [11]. SESN1 is considered to be a downstream factor of aerobic exercise, which tends to inhibit the activation of inflammatory signals, thus inhibiting the expression levels of inflammatory factors [12]. Increased SESN1 expression was found in neurons exposed to oxygen-glucose deprivation/reperfusion (OGD/R) treatment, and silencing of SESN1 makes neurons more susceptible to OGD/R damage, and upregulation of SESN1 improves OGD/R-induced neuronal damage by reducing apoptosis and production of ROS [13]. However, the possible effects of Sestrin on POCD were still unclear.

Nod-like receptor protein 3 (NLRP3) inflammasome is an intracellular protein complex that exacerbates neuroinflammation [14]. Several studies have demonstrated that NLRP3 is highly expressed in microglia and is involved in inflammasome formation through the recruitment of Apoptosis-associated speck-like protein containing a CARD (ASC) [14].

In this study, we investigated the role of SESN1 in POCD and found that it inhibits sevoflurane induced cognitive dysfunction, inhibits inflammation, oxidative stress and apoptosis,
and negatively regulates NLRP3. We consider this protein as a potential target for POCD.

2. Materials and methods

2.1 Cell culture and transfection

Primary neuron cells of rat hippocampus (Procell Life Science & Technology Co., Ltd.) were cultured with Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS) at 37 °C with 5% CO2. Cells were treated with 4% sevoflurane (1612540, purchased from sigma, Alexandria, VA, USA, purity >98%) for 6 h. Then transfection was performed using Lipofectamine 2000 (11668-027, Invitrogen, Carlsbad, CA, USA). The SESN1 plasmids were cloned in our lab by using the pcDNA3.1-vector. Serum deprivation was performed before the incubation with sevoflurane for 24 h.

2.2 Cell viability

Cells were plated into 96-well plates at the density of 1 × 10^3 cells/well. CCK-8 was added to cells following rinsing with PBS. Cells were incubated for 4 hours before the measurement of the Optical density (OD) 450 value using FACSCalibur flow cytometer (8.0, BD Biosciences, Inc., Franklin Lake, NJ, USA) and CellQuest Pro 5.1 (BD Biosciences, Inc., 8.0, Franklin Lake, NJ, USA).

2.3 Cell apoptosis

Annexin V/Propidium iodide (PI) apoptosis detection was conducted (Sigma Aldrich, USA). Cells were digested and mixed in reaction buffer containing Annexin V and PI for 5 min. Cell apoptosis were analyzed by a flow cytometer (Aria II, BD Biosciences, Franklin Lake, NJ, USA).

2.4 ELISA

The levels of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 in the medium were detected by ELISA kit. Samples were added into wells. Antibodies were added. Subsequently, enzyme substrate was added to develop color reaction.

2.5 Quantitative PCR

Trizol (15596-018, Invitrogen, Carlsbad, CA, USA) reagent were used for RNA extraction. Then RNA was reverse-transcribed into cDNA using Reverse Transcriptase (Promega, USA). Fast Start Universal SYBR Green Master kit (BL705A, Roche, Basel, Switzerland) was used for quantitative mRNA detection. The levels of targeted genes were determined by using the 2^{−ΔΔCT} method. The primers used were listed in Table 1.

2.6 ROS detection

For ROS staining, cells were mounted on glass coverslips and stained for dihydroethidium (DHE) as above. In independent experiment, DHE fluorescence of 20 cells per field in five random fields was quantified using AxioVision software (Carl Zeiss, Oberkochen City, Bartenfuburg Oblast, Germany).

2.7 Immunoblot assay

Radio Immunoprecipitation Assay (RIPA) buffer was used to fully lystate cells to extract protein. The equal amount of proteins from each sample was separated by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE), and further transferred onto the polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with Tris buffer solution (TBS) + Tween (TBST) containing 5% milk for 1 h. Subsequently, membranes were conjugated with primary antibodies targeting SESN1 (ab134091, 1:1000, Abcam, Cambridge, UK), Bax (ab32503, 1:1000, Abcam), Bcl-2 (ab32124, 1:1000, Abcam), NLRP3 (ab263899, 1:1000, Abcam), ASC (ab283684, 1:1000, Abcam), GSDMD-N (ab215203, 1:1000, Abcam), IL-18 (ab243091, 1:1000, Abcam), IL-1β (ab254360, 1:1000, Abcam), and β-actin (ab8226, 1:3000, Abcam) for 2 h. Subsequently the membranes were incubated with secondary antibodies for 1 h. The blots were analyzed with ECL kit.

2.8 Statistical analysis

GraphPad 5.0 software (Graphpad, La Jolla, CA, USA) was used for data analysis. Data were represented as mean ± standard deviation (SD). p < 0.05 was considered as statistically significant.

3. Results

3.1 SESN1 improves sevoflurane induced cell viability

To detect the effects of SESN1 on the progression of PCOD, we first constructed a cell model of PCOD via treating the rat hippocampal neurons with sevoflurane. We found that the high concentration of sevoflurane (SEV) (2%, 4%) suppressed the viability of neurons, whereas low concentration of SEV had modest effects on neuron viability (Fig. 1A). Therefore, the low concentration of SEV was used in the following experiments. Subsequently, SESN1 overexpression

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primers</th>
<th>Reverse primers</th>
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<tbody>
<tr>
<td>tumor necrosis factor (TNF)−α</td>
<td>5′-GAACCTGGCGAGAGGCACT−3′</td>
<td>5′-GGTCTGGGCCATAGAAGCT−3′</td>
</tr>
<tr>
<td>Interleukin (IL)-6</td>
<td>5′-CTGATGCTGGGCAACACC−3′</td>
<td>5′-CAGAATCTGCGATGTCAAC−3′</td>
</tr>
<tr>
<td>interleukin (IL)-1β</td>
<td>5′-TGGACCTTCCAGGATGAGGAC−3′</td>
<td>5′-GTTCACTCAGGGGCTTGTTA−3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-AGTATGACCTCCACTCAGGC−3′</td>
<td>5′-CACCAGTAGACTCCACGACA−3′</td>
</tr>
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GAPDH: glyceraldehyde-3-phosphate dehydrogenase.
Figure 1. SESN1 improves sevoflurane induced cell viability. (A) CCK-8 showed the effects of SEV (1%, 2% and 4%) on the cell viability. (B) Immunoblot showed the expression of SESN1 in cells upon the treatment of indicated drugs or plasmids. (C) The quantification of panel B. (D) CCK-8 showed the effects of SESN1 in cells upon the treatment of indicated drugs or plasmids. *, \( p < 0.05 \), ***, \( p < 0.001 \) SEV vs. control; ###, \( p < 0.001 \) SEV + SESN1 vs. SEV + vector. SESN1: Sestrin 1; SEV: sevoflurane.

Plasmids were transfected in hippocampal neurons. Through Immunoblot assays, we noticed that sevoflurane treatment increased the expression of SESN1 in hippocampal neurons (Fig. 1B,C). SESN1 overexpression further increased its expression levels in sevoflurane induced hippocampal neurons (Fig. 1B,C). Through CCK-8 assays, we found that SEV treatment suppressed the proliferation of hippocampal neurons, whereas SESN1 overexpression promoted the proliferation of sevoflurane-induced hippocampal neurons, compared to control (Fig. 1D). Therefore, SESN1 improves sevoflurane-induced hippocampal neuron viability.

3.2 SESN1 improves sevoflurane induced cellular inflammation

To explore the inflammatory response of SESN1 in SEV-induced hippocampal neurons, the level of IL-6, IL-1\( \beta \) and TNF-\( \alpha \) were measured in hippocampal neurons. We found that IL-6 was increased in SEV-induced hippocampal neurons, compared to control (Fig. 2A). SESN1 overexpression reduced these protein levels in SEV-induced hippocampal neurons, compared to control (Fig. 2A). The mRNA level of these proteins displayed the same pattern in SEV-induced hippocampal neurons, compared to control (Fig. 2B). These data implied that SESN1 improves sevoflurane induced cellular inflammation.

3.3 SESN1 improves sevoflurane induced oxidative stress

To examine the oxidative stress, 2',7'-Dichlorofluorescein (DCF) staining was used in each group. The data showed that the staining intensity was enhanced in SEV hippocampal neurons (Fig. 3). In addition, SESN1 overexpression reduced the DCF staining in SEV-induced hippocampal neurons, suggesting the inhibition of oxidative stress, compared to control (Fig. 3). These data suggested SESN1 improves sevoflurane induced oxidative stress.

3.4 SESN1 inhibits apoptosis of hippocampal neurons

We noticed the increased apoptotic cell proportion in SEV-induced hippocampal neurons. After SESN1 overexpression, the cell apoptosis was significantly reduced, compared to control (Fig. 4A). The levels of Bax and Bcl-2 were examined by Immunoblot. The level of Bax in the SEV treated group was
FIGURE 2. SESN1 improves sevoflurane induced cellular inflammation. (A,B) The level and mRNA level of IL-6, IL-1β and TNF-α in different groups were confirmed through ELISA (A) as well as qPCR (B) assays. ***, p < 0.001 SEV vs. control; #, p < 0.05, ##, p < 0.01, ###, p < 0.001 SEV + SESN1 vs. SEV + vector. SESN1: Sestrin 1; IL: interleukin; SEV: sevoflurane; TNF: tumor necrosis factor.

FIGURE 3. SESN1 improves sevoflurane induced oxidative stress. Immunostaining assays showed the ROS levels of cells upon the treatment of SEV or SESN1 plasmids. Scale bar indicates 50 µm. SESN1: Sestrin 1; SEV: sevoflurane.

3.5 SESN1 inhibits NLRP3 inflammasome activation

Subsequently, we detected the effects of SESN1 on the NLRP3 inflammasome activation of SEV-induced hippocampal neurons. Through the Immunoblot assays, we noticed that the expression of NLRP3 was increased in SEV treated hippocampal neurons (Fig. 5A), whereas SESN1 overexpression suppressed the expression of NLRP3, suggesting the inhibition of NLRP3 inflammasome (Fig. 5A). We further detected the expression of two key regulators of NLRP3 inflammasome, ACS and GSDMD-N. The results showed that the expressions of ACS and GSDMD-N were increased in SEV treated hippocampal neurons (Fig. 5B). Whereas SESN1 overexpression suppressed the expression of ACS and GSDMD-N (Fig. 5B). Furthermore, IL-1β and IL-18 expressions were upregulated in SEV treated hippocampal neurons, and decreased upon SESN1 overexpression (Fig. 5C). Therefore, SESN1 inhibits NLRP3 inflammasome activation.

4. Discussion

Postoperative cognitive dysfunction (POCD) is one of the most common postoperative complications [5]. The exact mechanism is still unclear. In recent years, studies have focused on the role of the inflammatory response induced by surgery and anesthesia in POCD [5]. The inflammatory
**FIGURE 4.** SESN1 inhibits apoptosis of hippocampal neurons. (A) Hippocampal neurons apoptosis in response to SESN1 and SEV were detected by Flow cytometry. The apoptosis cell percentage of cells upon the indicated treatment. (B) The expression of Bcl-2 and Bax in response to SESN1 and SEV in cells upon the indicated treatment was detected through Immunoblot assays. The quantification was conducted. ***, p < 0.01 SEV vs. control; ###, p < 0.01, ####, p < 0.001 SEV + SESN1 vs. SEV + vector. SESN1: Sestrin 1; PI: Propidium iodide; SEV: sevoflurane; FITC: Fluorescein isothiocyanate isomer I.

**FIGURE 5.** SESN1 inhibits NLRP3 inflammasome activation. (A) The expression of NLRP3 in response to SESN1 and SEV in cells upon the indicated treatment was detected through Immunoblot assays. The quantification was conducted. (B) The expression of ASC and GSDMD-N in response to SESN1 and SEV in cells upon the indicated treatment was detected through Immunoblot assays. The quantification was conducted. (C) The expression of IL-1β and IL-18 in response to SESN1 and SEV in cells upon the indicated treatment was detected through Immunoblot assays. The quantification was conducted. ***, p < 0.01 SEV vs. control; #, p < 0.05, ###, p < 0.001 SEV + SESN1 vs. SEV + vector. SESN1: Sestrin 1; NLRP: NOD-like receptor thermal protein domain associated protein; SEV: sevoflurane; ASC: Apoptosis-associated speck-like protein containing a CARD; IL: interleukin.
response in POCD is that astrocytes hinder nerve regeneration and release inflammatory factors. The inflammatory factor IL-1β can promote the release of neurotoxic substances, and cause the formation of hippocampal long term Potential (LTP) impairment through ROS, mitogen-activated protein kinase (MAPK) and other pathways [1]. Many studies have shown that the pathogenesis of postoperative cognitive impairment may be the result of multiple factors, and the inflammatory response induced by surgical trauma may play an important role in POCD [4]. Anti-inflammatory therapy may slow down POCD.

In this study, primary rat hippocampal neurons were treated with sevoflurane. Through CCK-8, ELISA, qPCR and Immunoblot, we noticed that SESN1 improved cell viability and inflammation induced by sevoflurane. Further through DCF and FCM assays, the results showed that SESN1 inhibited sevoflurane induced apoptosis and ROS production. Therefore, SESN1 could affect POCD progression. Sestrins are a highly conserved family of proteins produced in response to stress. There is only one Sestrin in vertebrates, and three Sestrins in vertebrates, namely Sestrin 1–3 [15]. Sertrin1 and Sestrin2 were found to reduce ROS levels and inhibit mammalian target of rapamycin C1 (mTORC1) activity in colorectal cancer cells, suggesting that Sestrin may slow the progression of related diseases by inhibiting these two molecules [16]. In recent years, the 3D molecular structure of Sestrin has been determined, and three functional sites have been revealed, namely, the active site that inhibits ROS, the active site that regulates mTOR, and the binding site of leucine [17]. Under oxidative stress, Sestrin is up-regulated by several transcription factors including p53, Nrf2, AP-1 and Foxos [18]. Overexpression of Sestrin renders cells resistant to oxidative stress, whereas lack of Sestrin renders cells and tissues highly vulnerable to oxidative stress [19]. Therefore, Sestrin is considered to be an important antioxidant defense molecule.

A moderate amount of reactive oxygen species (ROS) is necessary to maintain physiological balance; however, excessive accumulation of ROS leads to DNA and protein damage and accelerates the process of related diseases [20]. Studies have shown that reducing the abnormal elevation of ROS and MDA in the hippocampus of rats with cerebral ischemia/reperfusion injury can inhibit oxidative stress, thereby improving the POCD induced by cerebral ischemia/reperfusion injury [21]. Since we revealed the effects of SENS2 on the ROS of rat hippocampal neurons, the precise mechanism needs further study.

NLRP3-induced proinflammatory cytokines are associated with POCD, and NLRP3-induced reduction in neuroinflammation protects against cognitive impairment [22]. Notably, SESN1 inhibits NLRP3 inflammasome activation and foam cell formation [10]. Interestingly, we also revealed that SESN1 inhibited NLRP3 inflammasome activation, and further thought that SESN1 affected the progression of POCD via targeting NLRP3 inflammasome. In the following experiments, we will further investigate the expression profiles of hippocampal neuronal markers antineural fiber 200, neuron-specific enolase and microtubule-associated protein 2. To date, no inhibitors of SESN1 have been identified. Therefore, it is impossible to conduct inhibitor-related experiments, which is the direction of our current research.

5. Conclusions
In summary, SESN1 inhibited sevoflurane-induced cognitive dysfunction, inhibited inflammation, oxidative stress and apoptosis, and suppressed the formation of NLRP3 inflammasome.

AVAILABILITY OF DATA AND MATERIALS
All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS
LS—designed the research study. XYH—performed the research. YYL—analyzed the data. DLW—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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