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Knockdown of the Long Noncoding RNA CRNDE Ameliorates Apoptosis and Inflammation in Ischemia-Reperfusion-Induced Brain Injury via the mir-489-3p/FOXO3 Pathway

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ABSTRACT

AIM: To examine the role and mechanism of colorectal tumor differential expression (CRNDE) in brain injury induced by ischemic-reperfusion.

MATERIAL and METHODS: Sh-SY5Y cells were cultured, and oxygen and glucose deprivation/reperfusion (OGD/R) injury tests were performed. The effects on SH-SY5Y cells were evaluated by the Cell Counting Kit-8 (CCK-8) assay, qPCR, apoptosis analysis, western blot analysis, ELISA, a luciferase reporter assay, and an RNA pull-down assay.

RESULTS: Knockdown of CRBDE ameliorated SH-SY5Y cell impairment induced by OGD/R. CRNDE, the target of mir-489-3p, was directly bound to FOXO3. Mir-489-3p knockdown partially reversed OGD/R-mediated impairment in CRBDE knockdown SH-SY5Y cells.

CONCLUSION: The results indicate that knockdown of lncRNA CRNDE ameliorates apoptosis and the inflammatory response in ischemia-reperfusion-induced brain injury through the mir-489-3p/FOXO3 axis. LncRNA CRNDE may represent a novel therapeutic target for brain injury.

KEYWORDS: CRNDE, Ischemia-reperfusion, Apoptosis, mir-489-3p, FOXO3

INTRODUCTION

Stroke is a serious cerebrovascular disease (CVD) that impacts human health by causing long-term disability and death. In addition to the damage by ischemia itself, reperfusion can also lead to a cascade of pathological mechanisms that result in irreversible cerebral damage (10). Ischemia-reperfusion (IR) injury is the result of a prolonged ischemic insult followed by restoration of blood perfusion. It affects oxygen-dependent cells that rely on a continuous blood supply. IR contributes to stroke pathophysiology; therefore, reducing cerebral IR injury prevents stroke patients from hemorrhagic transformation and disruption of the blood-brain barrier (BBB) (40).

Long noncoding RNAs (lncRNAs) contain over 200 nucleotides. Although they are incapable of encoding functional proteins, lncRNAs play an important role in transcriptional and post-transcriptional gene regulation (24). The colorectal neoplasia differentially expressed (CRNDE) is a lncRNA located on chromosome 16 that exerts oncogenic effects in various cancers (30,43). Studies have revealed that the expression of CRNDE also increases in traumatic brain injury (TBI) patients. Furthermore, silencing CRNDE contributes to nerve restoration in a TBI rat model (37) and CRNDE exacerbates intrauterine infection-induced neonatal brain injury. CRNDE silencing may represent an effective cerebral protector (9). Moreover, CRNDE affects cell apoptosis and inflammation in several diseases (3,33,35). Nonetheless, there is still insufficient proof of CRNDE's role in ischemia injury, especially IR injury.

Mir-489-3p up-regulation in stroke protects neurons (13,14). In contrast, FOXO3 was upregulated in cerebrovascular disease induced by IR injury and modulated neuron survival, lineage

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commitment, stress response, and neuronal signaling (29). FOXO3 activation may induce autophagy, and FOXO3 knockdown may also protect against neuronal injury by inhibiting H_2O_2-induced damage (6). Another study revealed that miRNAs could indirectly regulate the FOXO3 function during cerebral ischemia/reperfusion injury (44). We demonstrated that CRNDE interacts with mir-489-3p, which should be associated with FOXO3 based on the Starbase algorithm. To elucidate the regulatory mechanisms underlying IR injury, we evaluated the association between CRNDE, mir-489-3p, and FOXO3. A cerebral ischemia cell injury model studied the expression of the molecules in SH-SY5Y cells following oxygen–glucose deprivation and reoxygenation (OGD/R). We determined the effect of CRNDE knockdown on injury to SH-SY5Y cells induced by OGD/R. Furthermore, inhibition of CRNDE revealed that CRNDE regulates OGD/R-mediated cell viability, apoptosis, and inflammation via the mir-489-3p/FOXO3 pathway.

MATERIAL and METHODS

Cell Culture

SH-SY5Y cells (Procell Life Science&Technology, Wuhan, China) were cultured in RPMI-1640 (PM150110) containing 1% P/S (PB180120) and 10% FBS (164210-500) in a humidified atmosphere with 95% air/5% CO_2 at 37°C. The cells were subcultured at a ratio of 1:3, and the medium was replaced three times each week.

Oxygen–Glucose Deprivation/Reperfusion (OGD/R) Model and Cell Transfection

After seeding the cells for 24 h, oxygen and OGD/R injury were carried out in OGD-medium for 0, 6, 12, and 24 h separately to mimic the ischemia-reperfusion process described in a previous study (22). The cells then returned to a normal environment. Because the expression of CRNDE was highest after 24 h of OGD/R induction, the experiments were performed under these conditions. Negative control (NC) inhibitor, NC mimics, and empty vectors were used as the corresponding NCs. In addition, mir-489-3p inhibitor and mir-489-3p mimics were transfected into SH-SY5Y cells. The 11 treatment groups were as follows:

(I) OGD/R model

(II) OGD/R model treated with si-NC (OGD/R + si-NC)

(III) OGD/R model treated with si-CRNDE (OGD/R + si-CRNDE)

(IV) Control group (con)

(V) OGD/R model treated with si-NC and NC inhibitor (OGD/R + si-NC + NC inhibitor)

(VI) OGD/R model treated with si-CRNDE and NC inhibitor (OGD/R + si-CRNDE + NC inhibitor)

(VII) OGD/R model treated with si-CRNDE and mir-489-3p inhibitor (OGD/R + si-CRNDE + mir-489-3p inhibitor)

(VIII) NC

(IX) mir-489-3p mimics

(X) NC inhibitor

(XI) mir-489-3p inhibitor

RNA Isolation and qRT-PCR

Total RNA was extracted from SH-SY5Y cells using a TRIzol one-step RNA extraction kit (TaKaRa Bio, Dalian, China) for the detection of lncRNA CRNDE and mir-489-3p. Subsequently, cDNA was synthesized from total RNA using the PrimeScript RT Reagent Kit (TaKaRa Bio, Dalian, China). The mRNA levels were determined relative to GAPDH expression, calculated using the $2^{-\Delta\Delta Ct}$ approach.

CCK-8 Assay

We performed a CCK-8 assay to evaluate SH-SY5Y cell growth in the various groups. SH-SY5Y cells (1×10^4/well) were seeded into 96-well plates for 48 h. Subsequently, 10 µL pf CCK-8 reagent (Abbkine, Wuhan, China) was added and incubated for 3 h. The absorbance (OD) value at 450 nm was measured using a microplate reader (Bio-Gene Technology, Guangzhou, China).

Flow Cytometry (FCM) Analysis of Cell Apoptosis

FCM analysis assessed SH-SY5Y cell apoptosis in the different groups. Cells were incubated with EdU (10 uM) for 2 h, fixed, and the Click-it reaction was done following the manufacturer's instructions. FCM was performed using FACSCalibur (BD Biosciences, Cytocell, SFR Francois Bonamy, Nantes, France) and analyzed with FlowJo software.

Western Blot Analysis

The expression levels of cleaved-caspase 3, bax, bcl-2, and FOXO3 in SH-SY5Y cells were determined by Western blot analysis based on a previous protocol (11).

Enzyme-Linked Immunosorbent Assay (ELISA)

In order measure IL-1β, IL-6, and TNF-α levels in the supernatant of SH-SY5Y cells, ELISA kits (MLBIO, China) were performed according to the manufacturer's instructions. OD values were measured at 450 nm using a microplate reader (Bio-Rad, USA).

Luciferase Reporter Assay

A luciferase assay determined whether mir-489-3p directly binds to the lncRNA CRNDE and/or FOXO3. After a 48-h transfection, we conducted luciferase assays using the Dual-Luciferase Reporter Assay System (Promega, WI).

RNA Pull-Down Assay

A biotinylated probe was bound to the junction area of CRNDE. A control probe (NC) was used, followed by 1×107 cells lysis. The CRNDE probe (Tsingke, Wuhan, China) was incubated with streptavidin magnetic beads (Prime Gene, Shanghai, China) for 2 h at room temperature. The cell lysates were incubated with probe-coated beads overnight at 4°C. After the beads were washed, the miRNAs were extracted with TRIzol reagent and examined by qRT-PCR.

Statistical Analysis

The data are expressed as the means ± standard deviations based on three replicates. Independent-samples t-tests or ANOVA were performed using SPSS 22.0 software to determine whether group differences were significant (IBM, Somers, NY, USA).

RESULTS

Knockdown of CRBDE Ameliorates SH-SY5Y Cell Impairment Induced by OGD/R

Based on qRT-PCR, CRNDE levels were significantly elevated ($p<0.001$) with increased OGD/R induction time. As shown in Figure 1, CRNDE expression in SH-SY5Y cells peaked after 24 hours of OGD/R induction. The expression of CRNDE was markedly lower after co-transfection with si-CRNDE compared with the con and OGD/R+si-NC groups. Cell proliferation was reduced following OGD/R induction but partially increased after co-transfection with si-CRNDE (OGD/R+si-CRNDE) based on a CCK-8 assay. In addition, apoptosis increased significantly in SH-SY5Y cells after OGD/R induction but partially decreased after co-transfection with si-CRNDE (OGD/R+si-CRNDE) as determined by flow cytometry. Western blot analysis revealed that bax and cleaved-caspase 3 were markedly elevated, and bcl-2 decreased in OGD/R-induced SH-SY5Y cells. The opposite results occurred after co-transfection with si-CRNDE. IL-1β, IL-6, and TNF-α expression increased in SH-SY5Y cell supernatants following OGD/R induction but partially decreased in OGD/R+si-CRNDE as determined by ELISA.

CRNDE Serves as a mir-489-3p Target

The subcellular localization of CRNDE was determined in SH-SY5Y cells using a karyoplasmic isolation assay. U6 and 18S were internal markers for the nucleus and cytoplasm, respectively. The expression of CRNDE in the nucleus and plasma was determined by qRT-PCR. As shown in Figure 2, CRNDE was primarily located in the cytoplasm. Based on the Starbase prediction algorithm, CRNDE targets mir-489-3p. qRT-PCR indicated that co-transfection of mir-489-3p mimics resulted in mir-489-3p overexpression in SH-SY5Y cells. Based on a luciferase reporter assay, compared with NC, mir-489-3p overexpression inhibited luciferase activity, and this effect was abolished following mutation at the predicted binding sites of mir-489-3p in SH-SY5Y cells. The RNA pull-down assay indicated that compared with the NC probe, the CRNDE probe significantly bound more mir-489-3p, which confirmed that CRNDE interacts directly with mir-489-3p. The results of qRT-PCR indicated that mir-489-3p expression was reduced in SH-SY5Y cells induced by OGD/R but partially increased after co-transfection of si-CRNDE.

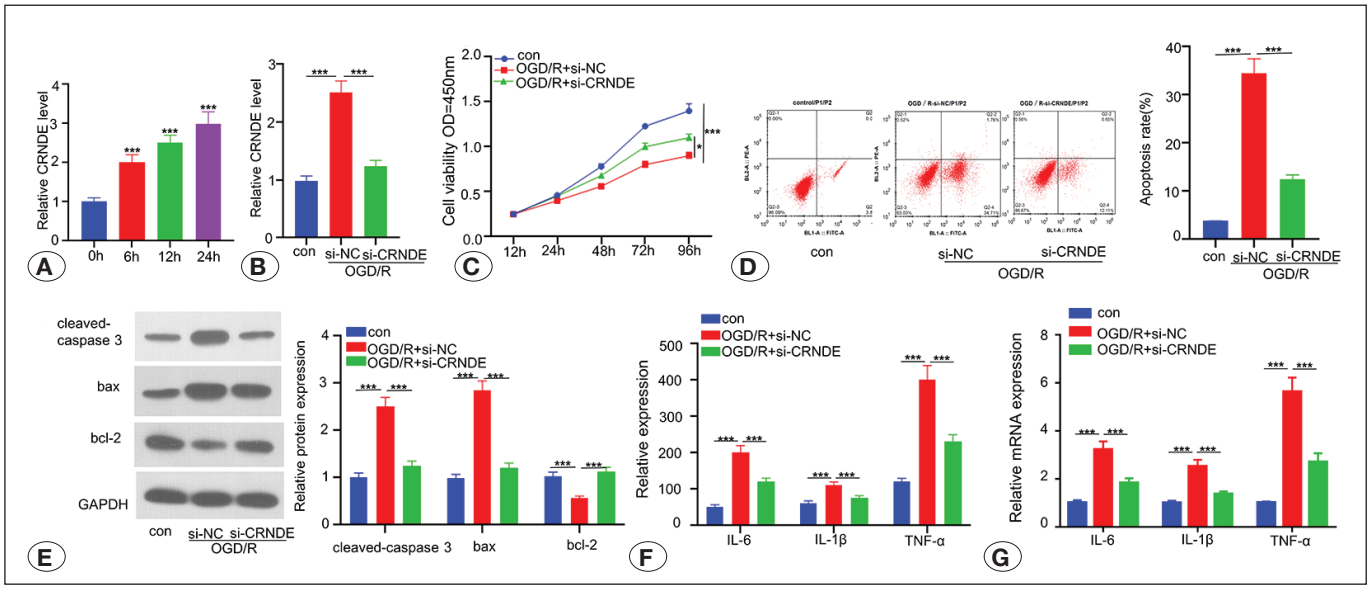

Figure 1: Knockdown of CRBDE ameliorates SH-SY5Y cell impairment induced by OGD/R. **A)** qRT-PCR revealed that the expression of CRNDE was gradually and significantly elevated ($p<0.001$) with increased OGD/R induction time. The content of CRNDE in SH-SY5Y cells was the highest after 24 h of OGD/R induction. **B)** qRT-PCR revealed that the expression of CRNDE was notably decreased after co-transfection of si-CRNDE compared with con and OGD/R+si-NC. **C)** Proliferation was reduced in SH-SY5Y cells induced by OGD/R but partially increased after co-transfection of si-CRNDE (OGD/R+si-CRNDE) in a CCK-8 assay. **D)** Apoptosis increased significantly in SH-SY5Y cells induced by OGD/R but partially decreased after co-transfection of si-CRNDE (OGD/R+si-CRNDE) as determined by flow cytometry. **E)** Western blot analysis revealed that cleaved-caspase 3 and bax increased significantly with decreasing bcl-2 in SH-SY5Y cells induced by OGD/R. The situation was reversed after co-transfection of si-CRNDE. **F)** ELISA revealed increased expression of IL-6, IL-1β, and TNF-α in SH-SY5Y cell supernatants induced by OGD/R, but partially decreased in OGD/R+si-CRNDE. **G)** qRT-PCR revealed increased expression of IL-6, IL-1β, and TNF-α in SH-SY5Y cells induced by OGD/R, but partially decreased after co-transfection of si-CRNDE.

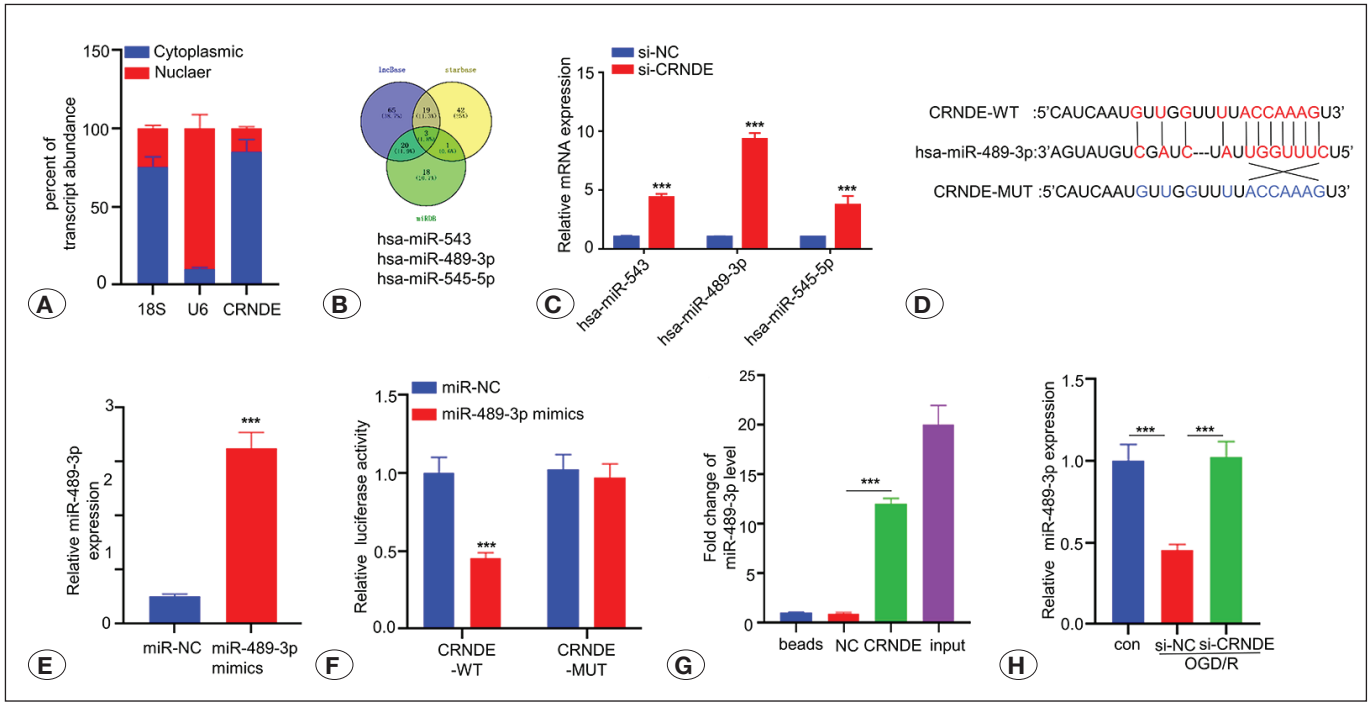

Figure 2: CRNDE is a target of mir-489-3p. **A)** The subcellular localization of CRNDE was detected in SH-SY5Y cells using a karyoplasmic isolation assay. U6 and 18S were used as internal markers of the nucleus and cytoplasm, respectively. The expression of CRNDE in the nucleus and plasma was detected by qRT-PCR. CRNDE was primarily localized to the cytoplasm. **B)** According to the Starbase prediction algorithm, CRNDE targets mir-489-3p. **C)** qRT-PCR indicated that co-transfection of mir-489-3p mimics overexpressed mir-489-3p in SH-SY5Y cells. **D)** A luciferase reporter assay revealed that compared with NC, overexpression of mir-489-3p inhibited luciferase activity in the cells. The effect reversed after mutation of the predicted binding sites of mir-489-3p in SH-SY5Y cells. **E)** The RNA pull-down assay showed that compared with the NC probe, the CRNDE probe significantly bound more mir-489-3p, which confirmed that CRNDE directly interacts with mir-489-3p. **F)** Based on qRT-PCR, the expression of mir-489-3p was reduced in SH-SY5Y cells following OGD/R induction, but partially increased after co-transfection of si-CRNDE. **G)** RNA pull-down assay showing the CRNDE probe bound significantly more mir-489-3p compared with the NC probe. **F)** qRT-PCR revealed that mir-489-3p expression was reduced in SH-SY5Y cells induced by OGD/R but partially increased after co-transfection of si-CRNDE.

Knockdown of mir-489-3p Partially Reverses SH-SY5Y Cell Impairment Induced by OGD/R Knockdown of CRNDE

The expression levels of mir-489-3p were markedly decreased following co-transfection of the mir-489-3p inhibitor compared with the NC inhibitor (Figure 3). A CCK-8 assay revealed that SH-SY5Y cell proliferation induced by OGD/R was decreased, but partially increased after co-transfection of si-CRNDE, which was partially reduced after co-transfection of the mir-489-3p inhibitor. Flow cytometry analysis revealed that apoptosis increased significantly in SH-SY5Y cells following OGD/R induction but partially decreased after co-transfection of si-CRNDE (OGD/R+si-CRNDE), which partially increased after co-transfection of mir-489-3p inhibitor. A western blot analysis revealed that bax and cleaved-caspase 3 expression were markedly elevated; however, bcl-2 expression decreased in SH-SY5Y cells induced by OGD/R. The results were opposite following co-transfection of si-CRNDE, but recovered after mir-489-3p inhibitor co-transfection. Based on ELISA, IL-1β, IL-6, and TNF-α expression increased in SH-SY5Y cells following OGD/R induction but partially decreased in OGD/R+si-CRNDE. The results were reversed again after co-transfection with the mir-489-3p inhibitor.

Mir-489-3p a Target of FOXO3

The Starbase algorithm predicted a mir-489-3p binding site within the 3'-UTR of FOXO3. Based on a luciferase reporter assay, compared with NC, mir-489-3p overexpression inhibited luciferase activity, and this effect was abolished after mutation of the predicted FOXO3 3'-UTR binding site. Compared with SH-SY5Y cells co-transfected with NC and mir-489-3p mimics, a Western blot analysis suggested that mir-489-3p mimic co-transfection markedly down-regulated FOXO3 (Figure 4). Compared with SH-SY5Y cells co-transfected with the mir-489-3p and NC inhibitors, the mir-489-3p inhibitor significantly restored FOXO3 levels. A western blot analysis revealed that FOXO3 expression increased markedly in SH-SY5Y cells induced by OGD/R but partially decreased after co-transfection of si-CRNDE. Co-transfection with the mir-489-3p inhibitor significantly increased FOXO3 expression.

Overexpression of FOXO3 Partially Reverses the Effect of mir-489-3p Silencing on OGD/R-Mediated SH-SY5Y Cell Damage

To determine the regulatory effect of mir-489-3p on FOXO3 concerning OGD/R-mediated SH-SY5Y cell damage, we overexpressed FOXO3 in SH-SY5Y cells. FOXO3 protein

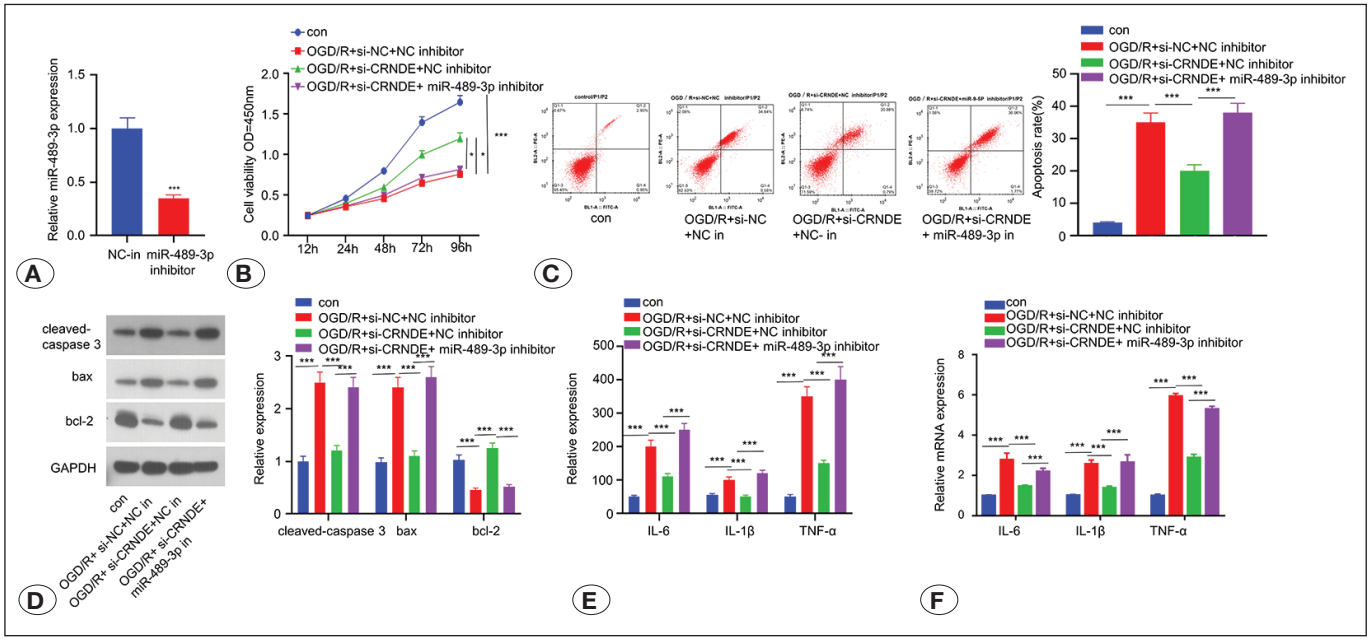

Figure 3: Knockdown of mir-489-3p partially reverses SH-SY5Y cell impairment resulting from OGD/R knockdown of CRNDE. **A)** The expression levels of mir-489-3p reduced markedly after co-transfection of the mir-489-3p inhibitor compared with the NC inhibitor. **B)**A CCK-8 assay revealed that the proliferation of PD 12 cells induced by OGD/R decreased, partially increased after co-transfection of si-CRNDE, and partially reduced after co-transfection of the mir-489-3p inhibitor. **C)** Flow cytometry analysis revealed that apoptosis was significantly increased in SH-SY5Y cells induced by OGD/R, partially decreased after co-transfection of si-CRNDE (OGD/R+si-CRNDE), and partially elevated after co-transfection of the mir-489-3p inhibitor. **D)** Western blot analysis indicated that cleaved-caspase 3 and bax increased notably, but bcl-2 decreased in SH-SY5Y cells induced by OGD/R. The result was the opposite after co-transfection of si-CRNDE and reversed after co-transfection of the mir-489-3p inhibitor. **E)** ELISA revealed that IL-6, IL-1β, and TNF-α expression increased in SH-SY5Y cells following OGD/R induction but partially decreased in OGD/R+si-CRNDE. The results were reversed after the co-transfection of the mir-489-3p inhibitor. **F)** qRT-PCR results of IL-6, IL-1β, and TNF-α expression in SH-SY5Y cells showed similar change pattern among the groups.

levels increased significantly after transfection with a FOXO3 overexpression plasmid (Figure 5, $p<0.01$). First, a decreased cell proliferation occurred in OGD/R-induced SH-SY5Y cells, which increased partially after mir-489-3p co-transfection. This recovery was ameliorated by co-transfection with FOXO3 and mir-489-3p in SH-SY5Y cells ($p<0.01$). Second, the increased proportion of OGD/R-induced SH-SY5Y cell apoptosis was partially reduced following subsequent mir-489-3p co-transfection. The recovery was attenuated by co-transfection with FOXO3 and mir-489-3p in SH-SY5Y cells ($p<0.01$). Consistently, increased IL-1β, IL-6, and TNF-α expression was observed in SH-SY5Y cells following OGD/R induction but partially decreased after transfection with mir-489-3p. However, IL-1β, IL-6, and TNF-α expression partially increased after co-transfection of mir-489-3p with FOXO3 ($p<0.01$). Finally, elevated IL-1β, IL-6, and TNF-α mRNA expression observed in SH-SY5Y cells after OGD/R treatments partially decreased after co-transfection with mir-489-3p. IL-1β, IL-6, and TNF-α mRNA expression partially increased after co-transfection of FOXO3 and mir-489-3p ($p<0.01$).

DISCUSSION

This is the first study to explore the effect of the noncoding RNA, CRNDE, on ameliorating apoptosis and inflammation resulting from brain impairment induced by IR through the mir-489-3p/FOXO3 signaling pathway. We used qRT-PCR to demonstrate that CRNDE expression was gradually increased with OGD/R induction time. However, following CRNDE knockdown, SH-SY5Ycell proliferation increased, and apoptosis decreased. In addition, IL-1β, IL-6, and TNF-α expression was partially decreased, which indicated a reduction in inflammation.

CRNDE was considered upregulated in colorectal cancer (7,16), and higher expression occurred in other solid tumors (15,27). CRNDE exerts an oncogenic effect in brain tumors (2). It has been implicated in multiple tumor-associated processes, such as cell growth, invasion, and metastasis. It also induces EMT, alters the tumor immune microenvironment, inhibits apoptosis (41), and causes inflammation to modulate the tumorigenesis of glioma (17). Increased CRNDE was also associated with TBI. A recent study showed that CRNDE up-regulation caused WI-38 cell injuries, significantly inhibiting cell viability, promoting cell apoptosis, and increasing the levels of inflammatory cytokines (45). In addition, siRNA-mediated downregulation of CRNDE enhanced neuronal activity and suppressed inflammatory factors and inhibited neuronal apoptosis of rats with TBI (6). It also ameliorated apoptosis in neonatal rats with intrauterine infection-caused brain injury (8). The results of these studies are consistent, in part, with that of our study.

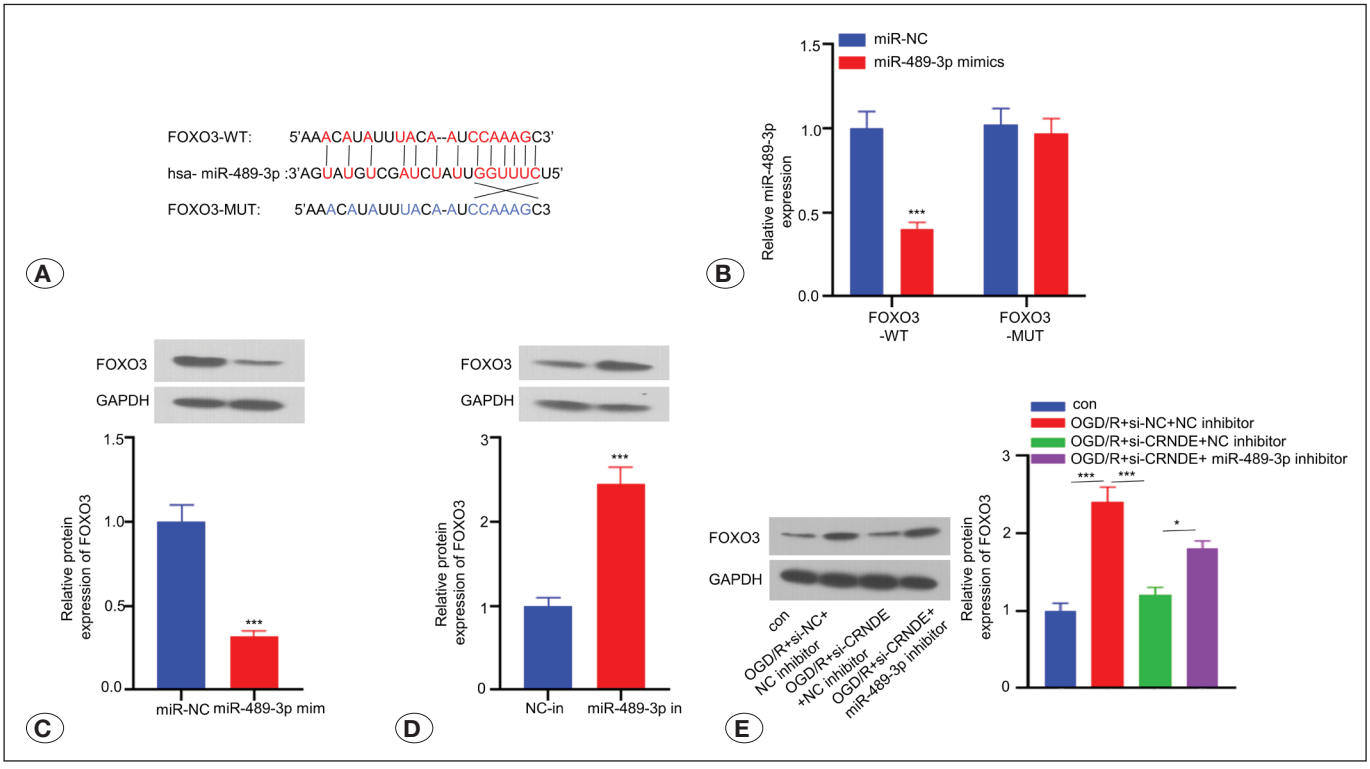

Figure 4: Mir-489-3p is a target of FOXO3. **A)** Based on the Starbase prediction algorithm, there is a binding site for mir-489-3p in the noncoding 3'-UTR of FOXO3. **B)** A luciferase reporter assay revealed that compared with NC, mir-489-3p overexpression inhibited luciferase activity in cells, but restored after mutation of the predicted FOXO3 3 '-UTR binding site. **C)** A comparison of SH-SY5Y cells co-transfected with NC and mir-489-3p mimics revealed that the mir-489-3p mimics significantly decreased the expression of FOXO3. **D)** A comparison of SH-SY5Y cells co-transfected with NC inhibitor and mir-489-3p inhibitor revealed that the mir-489-3p inhibitor markedly enhanced the expression of FOXO3. **E)** Western blot analysis indicated that the expression of FOXO3 increased markedly in SH-SY5Y cells induced by OGD/R but partially decreased after co-transfection of si-CRNDE. Co-transfection of the mir-489-3p inhibitor significantly increased the expression of FOXO3.

In the present study, the change in cleaved-caspase-3, bax, and bcl-2 indicated increased apoptosis in SH-SY5Y cells. Cleaved-caspase-3 is responsible for significant proteolysis during apoptosis and is an established marker of apoptotic cell death (4). The bcl-2 family promotes or inhibits apoptotic signaling triggered by damaged mitochondria (25,32,38). The Bcl-2/Bax/Cleaved-caspase-3 apoptotic signaling pathways can attenuate apoptosis and survival, which is involved in many nervous system diseases. TNF-α, IL-1β, and IL-6 are inflammatory markers in cells. Following brain injury, similar to ischemic stroke, resident microglial cells are inflammatory cells involved in the damage response. Microglia exert neurotoxic effects on ROS production via NADPH oxidase (28), cytokines (TNF-α, IL-1β and IL-6) (28), and MMP-9 (5). Therefore, we verified that noncoding RNA CRNDE could ameliorate apoptosis and inflammation following brain injury induced by IR. Preventing and reversing cerebral IR injury by alleviating apoptosis and inflammation is a potentially novel therapeutic goal.

We discovered an association between CRNDE and mir-489-3p and an interaction between mir-489-3p with FOXO3 using the Starbase prediction algorithm, which was further verified by luciferase reporter assays, and RNA pull-down assays. Mir-489-3p is considered a brain-specific miRNA that is highly expressed in CH rats' hippocampus and is a multi-functional regulator of neurogenesis (21). In previous animal experiments, mir-489-3p expression inhibited glioblastoma cell growth, invasion, migration, and induced apoptosis (42). In addition, mir-489-3p expression significantly decreased multiple myeloma cell proliferation (34). Moreover, up-regulation of mir-489-3p may play a significant role in congenital hypothyroidism (21). Also, mir-489-3p expression inhibited GC growth, invasion, and migration (39). Mir-489-3p decreased in cultured neurons following OGD/R induction, and downregulation of mir-489-3p reversed SH-SY5Y cell impairment induced by OGD/R knockdown of CRNDE illustrates the protective effect of mir-489-3p during cerebral IR injury.

FOXO3 is a transcription factor that regulates various genes and biological processes (12). An imbalance in FOXO3 is relevant to a variety of cellular processes, including metabolism (20), autophagy (19), proliferation, and apoptosis (36). FOXO3 may be overexpressed in inflammation and other stress responses (41), and it functions in anti-oxidation and premature aging of the enteric nervous system (1). MiRNAs that target FOXO3 have the potential to regulate apoptosis in cancer (18), acute kidney injury (23), and renal fibrosis (31). FOXO3 pro-

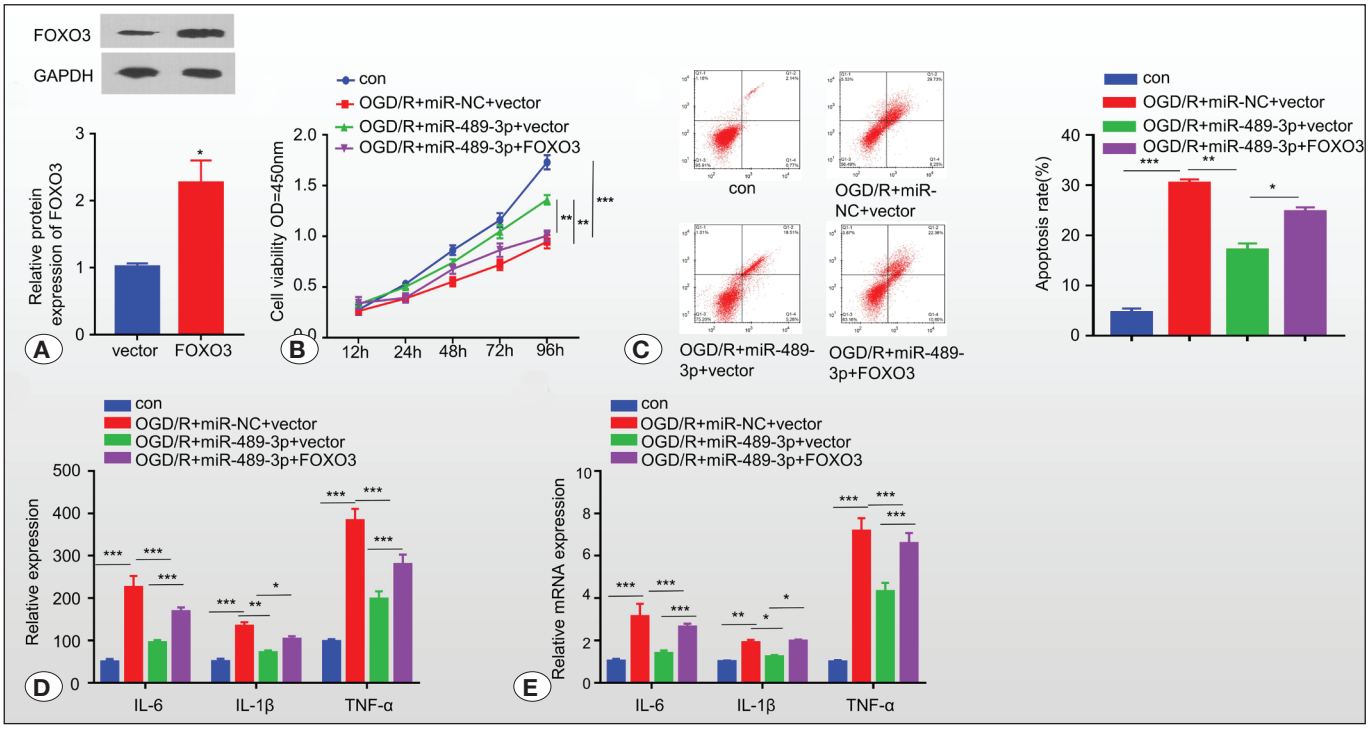

Figure 5: Overexpression of FOXO3 partially reverses the effect of mir-489-3p knockdown on OGD/R-induced SH-SY5Y cell damage. **A)** The protein expression levels of FOXO3 in SH-SY5Y cells from various groups (vector, FOXO3) as detected by Western blot. **B)** A CCK8 assay was used to measure the proliferation of SH-SY5Y cells in the different groups (CON, OGD/R + mir-NC + vector, OGD/R + mir-489-3p + vector, OGD/R + mir-489-3p + FOXO3). **C)** Flow cytometry was used for the detection of apoptosis in SH-SY5Y cells from the various groups (CON, OGD/R + mir-NC + vector, OGD/R + mir-489-3p + vector, OGD/R + mir-489-3p + FOXO3). **D)** ELISA was used to identify IL-1β, IL-6, and TNF-α in different groups of SH-SY5Y cells (CON, OGD/R + mir-NC + vector, OGD/R + mir-489-3p + vector, OGD/R + mir-489-3p + FOXO3). **E)** qRT-PCR was used to measure the levels of IL-1β, IL-6, and TNF-α in SH-SY5Y cells from the various groups (CON, OGD/R + mir-NC + vector, OGD/R + mir-489-3p + vector, OGD/R + mir-489-3p + FOXO3).

motes inflammation induced by IR and suppresses the activation of apoptotic signaling. Our results indicate that mir-489-3p is directly bound to FOXO3, which is consistent with the results of previous studies.

CONCLUSION

Our results indicate that knockdown of the long noncoding RNA, CRNDE, ameliorates cerebral neuron apoptosis and inflammation impairment induced by IR through the mir-489-3p/FOXO3 axis. CRNDE may represent a novel therapeutic target for the treatment of IR.

AUTHORSHIP CONTRIBUTION

Study conception and design: ML

Data collection: YH

Analysis and interpretation of results: YH

Draft manuscript preparation: ML

Critical revision of the article: YH, ML

All authors (YH, ML) reviewed the results and approved the final version of the manuscript.

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