



Silencing of circFoxO3 Protects HT22 Cells from Glutamate-Induced Oxidative Injury via Regulating the Mitochondrial Apoptosis Pathway

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Abstract

Recent studies demonstrated that FoxO3 circular RNA (circFoxO3) plays an important regulatory role in tumorigenesis and cardiomyopathy. However, the role of circFoxO3 in neurodegenerative diseases remains unknown. The aim of this study was to examine the possible role of circFoxO3 in neurodegenerative diseases and the underlying mechanisms. To model human neurodegenerative conditions, hippocampus-derived neurons were treated with glutamate. Using molecular and cellular biology approaches, we found that circFoxO3 expression was significantly higher in the glutamate treatment group than that in the control group. Furthermore, silencing of circFoxO3 protected HT22 cells from glutamate-induced oxidative injury through the inhibition of the mitochondrial apoptotic pathway. Collectively, our study demonstrates that endogenous circFoxO3 plays a key role in inducing apoptosis and neuronal cell death and may act as a novel therapeutic target for neurodegenerative diseases.

Keywords Circular RNA · FoxO3 · Neurodegenerative · Reactive oxygen species · Apoptosis · Neuroprotection

Introduction

With the increase in the proportion of the aged population, the prevalence of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and motor neuron disease (MND), has significantly increased (Tidwell et al. 2017). Neurodegenerative diseases are irreversible, resulting in progressive cell degeneration and loss, and represent a major health burden due to inefficient treatment strategies (Rotermund et al. 2018). Consequently, it is important to find an effective treatment. Neuronal apoptosis caused by oxidative stress serves as an underlying

mechanism of many neurodegenerative diseases and, thus, represents a potential therapeutic target (Chakrabarti and Mohanakumar 2016).

Circular RNA (circRNA) is a unique type of endogenous non-coding RNA (ncRNA) (Memczak et al. 2013) that, unlike traditional linear RNA, has a closed ring structure without a 5' cap structure and a 3' tail structure (Taulli et al. 2013). CircRNAs are resistant to RNase R and, thus, they are more stable than linear RNA in blood and tissues (Zhao et al. 2019). Recent studies have shown that a novel circRNA called circFoxO3 plays an important regulatory role in myocardial senescence and serves as a therapeutic target (Li et al. 2015). CircFoxO3 is derived from the forkhead box-containing protein, class O 3 (FoxO3) gene (Du et al. 2016). CircFoxO3 acts as an enhancer for the FoxO3 gene and leads to apoptosis (Yang et al. 2016). Increased expression of circFoxO3 is observed in the heart tissue derived from aged mice and humans and can be a potential target for the treatment of various age-related heart diseases (Du et al. 2017). However, the links between circFoxO3 and neurodegenerative diseases have not been explored. In the present study, we established a model of neuronal oxidative stress in HT22 cells induced by glutamate and elucidated the previously unrecognized role

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and the mechanism underlying the action of circFoxO3 in neurodegenerative diseases.

Materials and Methods

Cell Culture

Mouse hippocampal HT22 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone, USA) containing 10% foetal bovine serum (FBS) (HyClone) and 1% penicillin and streptomycin (Sigma-Aldrich, USA).

Cell Viability Assay

Calcein-acetyoxymethyl (Calcein-AM) (Anaspec, USA) and Propidium Iodide (PI) (Invitrogen, USA) kits were used for Calcein-AM/PI double staining, using our previously published protocol (Lin et al. 2018) with minor modifications. Briefly, the cells were incubated with 1 µg/ml Calcein-AM and 1 µg/ml PI for 15 min at 37 °C. After washing with phosphate buffered saline (PBS), the cells were observed by a fluorescence microscope (The EVOS® FL Auto Imaging System, Thermo Scientific, USA).

Cell viability was assessed by using Calcein-AM to stain the live cells. The fluorescence intensity of Calcein-AM was measured using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, USA) at 485/530-nm excitation/emission wavelengths. The percentage of cell viability was determined by normalizing the data in the experimental group to that in the control group.

Quantitative Real-Time PCR (qPCR) and circRNA Sequencing

Total RNA was isolated using TRIzol® reagent (Invitrogen) as previously described (Pan et al. 2019). Total RNA was reverse transcribed to synthesize cDNA using SuperScript III® (Invitrogen) according to the manufacturer's protocol. The relative expression of circFoxO3 was determined using SYBR Green qPCR SuperMix (Invitrogen) in the ViiA™ 7 Real-time PCR System (Applied Biosystems, USA). The primers used for the qPCR analysis of circFoxO3 were forward: 5'-CGTTGTTGGTTTGAATGTGGG-3', reverse: 5'-TGCCGGATGGAGTTCTGCT-3'. β-actin (forward: 5'-GTACCACCATGTACCCAGGC-3', reverse: 5'-AACGCAGCTCAGTAACAGTCC-3') was used as an internal standard control. Direct PCR product Sanger sequencing was performed by Majorbio Biotech Co., Ltd. (Shanghai, China).

RNA Interference (RNAi)

CircFoxO3 targeting small interfering RNA (siRNA) molecules were chemically synthesized by GenePharma Company (GenePharma, China). The sequence of the specific circFoxO3 siRNA is shown in Fig. 2a. The sequence of the scrambled siRNA was UUCUCCGAACGUGUCACGUTT. The siRNA molecules were transfected into HT22 cells with Lipofectamine iMAX (Invitrogen) for 24 h. After transfection, the HT22 cells were exposed to glutamate and then subjected to various examinations.

Fluorescent In Situ Hybridization (FISH)

To measure circFoxO3 expression in HT22 cells, a FISH assay was performed with a 5'Cy3-labelled circFoxO3 probe (GenePharma). A scramble sequence labelled with 5'Cy3 was used as a negative control (GenePharma).

The FISH assay was performed using a previously described procedure (Bi et al. 2019). In brief, HT22 cells were seeded in a 96-well microplate at a density of 5,000 cells per well. After RNAi and glutamate treatment, the HT22 cells were fixed by immersing the cells in absolute ethanol for 15 min. The cells were then permeabilized with 0.1% Triton X-100 for 15 min. After dehydration with ethanol, the probe mixture was added to each well and denatured at 73 °C for 5 min followed by overnight hybridization at 37 °C in an incubator. After annealing and renaturation, a hybrid of the target nucleic acid probe was formed, and the fluorescent signal was detected.

Immunocytochemistry Staining

Immunocytochemistry was performed as described previously (Pan et al. 2015). Briefly, the cells were fixed in BD Cytofix/Cytoperm solution (BD Biosciences) and permeabilized using 0.1% Triton X. The cells were incubated overnight in primary antibodies targeting FoxO3 (Cell Signaling Technology, Danvers, USA, 1:50, #2497), Bim (Cell Signaling Technology, 1:100, #2933) and Cleaved caspase-3 (Cell Signaling Technology, 1:100, #9661) followed by labelling with Cy3 goat anti-rabbit IgG (Beyotime, Shanghai, China). Then, the cells were further incubated with 0.5 mg/ml DAPI to stain the nuclei. Images were obtained using a confocal microscope (Zeiss, Thornwood, NY, USA) or fluorescence microscope.

TdT-Mediated dUTP Nick end Labelling (TUNEL) Staining

The HT22 cells were seeded in a 96-well microplate at a density of 5000 cells per well. After RNAi and glutamate treatment, the HT22 cells were fixed by immersing the cells in 4% methanol-free formaldehyde solution in PBS for 20 min. The cells were then permeabilized with 0.5% Triton X-100 for 5 min. The cells were labelled with fluorescein TUNEL reagent mixture for 60 min at 37 °C in the dark to avoid exposure to light, according to the manufacturer's instructions (Promega, USA). After DAPI staining, images were obtained using a fluorescence microscope.

Western Blot Analysis

Western blot analysis was performed on the cell lysates according to standard protocols, as described previously (Zhang et al. 2017; Pan et al. 2017; Guo et al. 2019). Antibodies recognizing Bim (1:1000, #2933), Cytochrome c (1:1000, #11,940), Caspase-3 (1:1000, #9665), FoxO3 (1:1000, #2497) and β -Actin (1:1000, #4970) were purchased from Cell Signaling Technology.

Measurement of Reactive Oxygen Species (ROS)

The levels of intracellular ROS were evaluated using the 2',7'-dichlorofluorescein diacetate (H2DCFDA) assay kit (Invitrogen), according to a previously published protocol (Pan et al. 2014). Briefly, the cells were loaded with 10 μ mol/L H2DCFDA for 20 min at 37 °C in the dark. The fluorescence intensity of the cells was measured with 530/485-nm excitation/emission wavelengths using fluorescence microscopy.

Measurement of Mitochondrial Membrane Potential (MMP)

The MMP was measured using the 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1) dye (Beyotime). The cells were incubated with 5 μ g/ml JC-1 working solution for 30 min at 37 °C. The normal mitochondria emitted strong red fluorescence, whereas the mitochondria with decreased MMP emitted green fluorescence. The red and green fluorescence intensities were measured with Image-Pro Plus software (Media Cybernetics, USA). The loss of MMP is indicated by an increase in the ratio of green/red fluorescence intensity.

Statistical Analysis

All the experiments were performed at least in triplicate. The data are presented as the mean \pm standard error of mean

(SEM). Graph Pad Prism 5.0 (GraphPad software, USA) software was used for statistical analysis. Differences among different groups were analysed using one-way ANOVA followed by Tukey's post hoc analysis. A *p* value of less than 0.05 was considered statistically significant.

Results

CircFoxO3 is Involved in Glutamate-Induced HT22 Injury

It has been reported that circFoxO3 is highly expressed in aged heart tissue (Du et al. 2017). We first determined the expression level of circFoxO3 in HT22 neuronal cells and found that circFoxO3 expression was higher in the glutamate treatment group than that in the control group.

Based on the relevant literature (Lin et al. 2018; Jin et al. 2018; Honrath et al. 2018), we used different concentrations of glutamate (6–10 mM) to test the efficacy. Calcein-AM/PI staining and immunofluorescence quantification showed that 6–10 mM glutamate treatment induced HT22 cell death in a dose-dependent manner (Fig. 1a, b). We next determined whether circFoxO3 is expressed in HT22 cells using qPCR. Using the Sanger sequencing approach, we confirmed that circFoxO3 was specifically amplified by qPCR in our study. The backsplice junction sequences were completely consistent with circBase (<https://www.circbase.org>) (Fig. 1d). As shown in Fig. 1c, our qPCR analysis showed that glutamate increased circFoxO3 expression in a concentration-dependent manner, suggesting that the over-expression of circ-FoxO3 was associated with glutamate-induced HT22 injury.

Blocking circFoxO3 Protects HT22 Cells Against Glutamate-Induced Injury

To investigate the role of circFoxO3 in glutamate-induced neuronal death, we inhibited the expression of circFoxO3 by transfecting siRNA into HT22 cells. Two siRNAs were designed according to the backsplice junction sequence and transfected into HT22 cells to knock down circFoxO3 expression. The two siRNAs specifically silenced circFoxO3 without impacting FoxO3 mRNA expression (Fig. 2a). The transfection efficiency was high in the HT22 cell line (Fig. 2a). To verify the knockdown effect of siRNA against circFoxO3, the expression levels of circFoxO3 were examined by qPCR. Both siRNAs significantly decreased the expression levels of circFoxO3 compared with the levels observed in the siRNA negative control group ($p < 0.05$) (Fig. 2b). The siRNA-1 treatment led to a 50% reduction in the expression of circFoxO3 and significantly reduced the expression levels of the FoxO3 protein in HT22 cells.

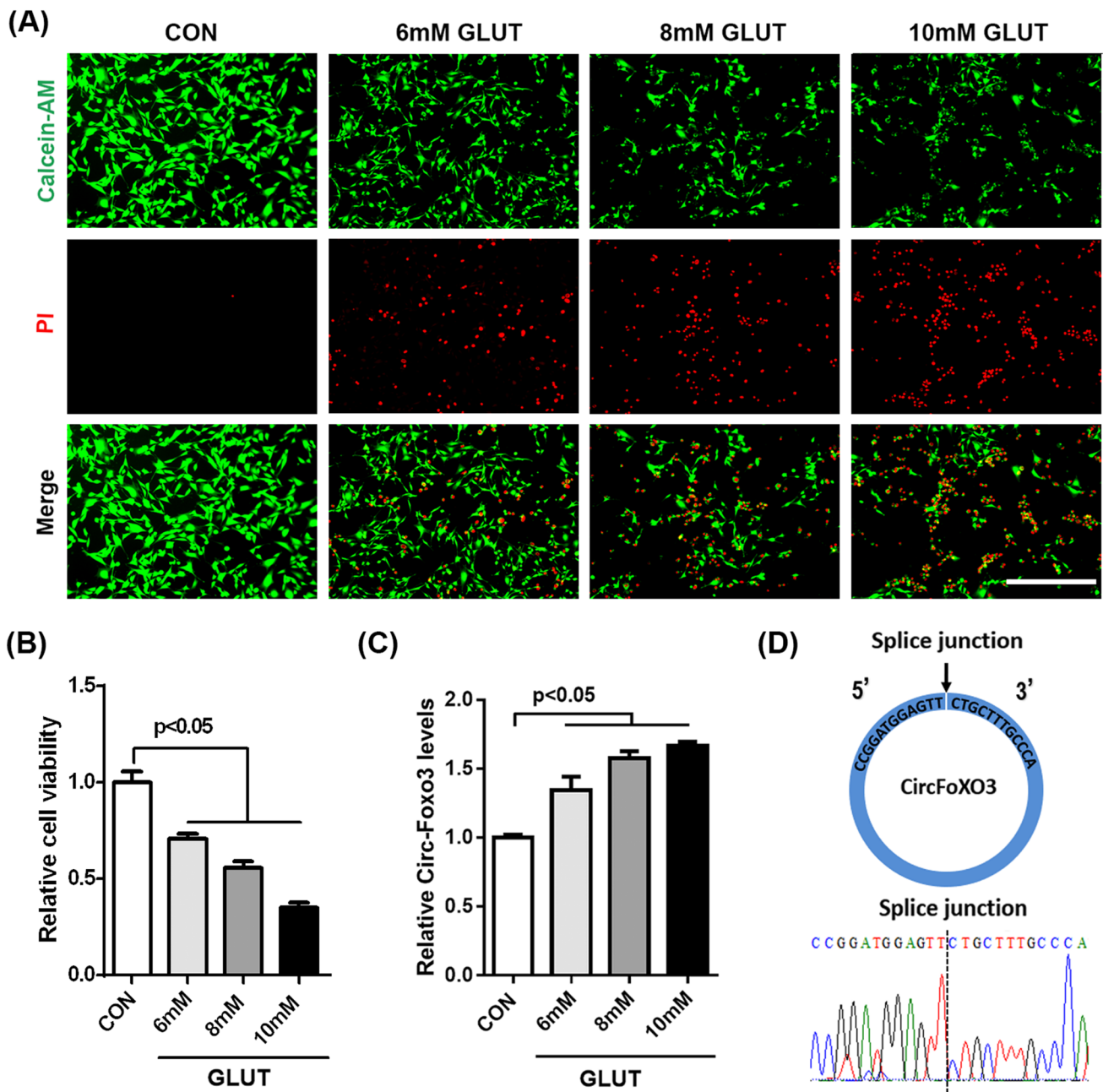


Fig. 1 Involvement of circFoxO3 in glutamate-induced HT22 injury. **a** Cells were treated with glutamate for 24 h. The live and dead cells were observed using Calcein-AM and PI staining, respectively. Scale bar=400 μ m. **b** A Calcein-AM cell viability assay showed that glutamate treatment significantly decreased the cell survival rate in a

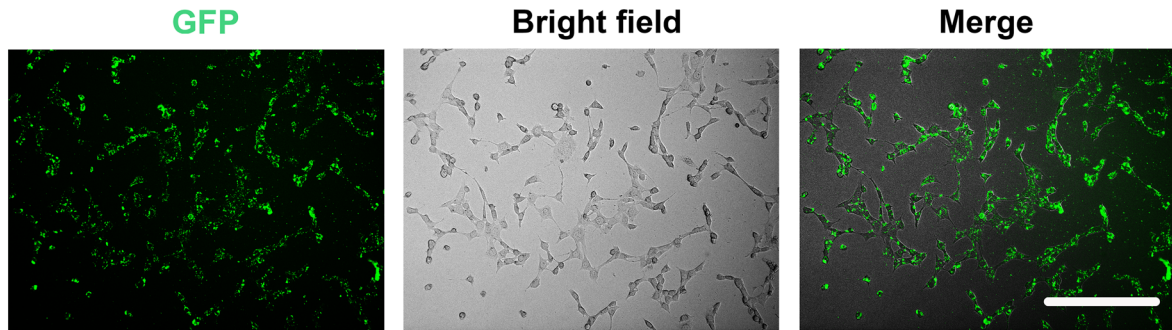
dose-dependent manner. **c** qPCR showed that glutamate treatment increased the expression levels of circFoxO3 in a dose-dependent manner. **d** Schematic diagram illustrating the structure of circFoxO3. The backsplice junction sequences of circFoxO3 were validated by Sanger sequencing

Therefore, siRNA-1 was chosen as the optimum interference and was denoted as siRNA in further experiments.

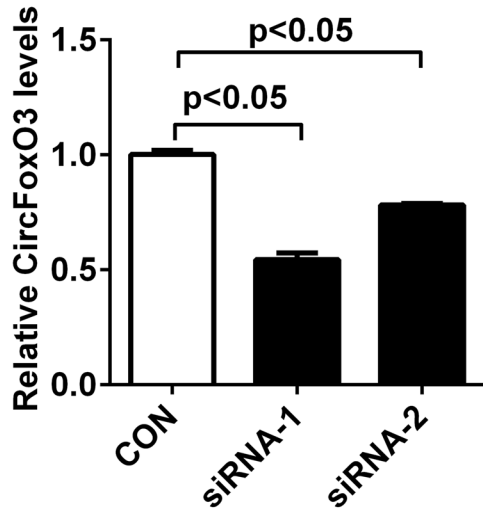
A Calcein-AM cell viability assay demonstrated that siRNA targeting circFoxO3 protected HT22 cells from glutamate-induced injury (Fig. 3a, b). FISH staining was used to observe the intracellular expression of circFoxO3.

The designed probe appeared to be specific for circFoxO3 (Fig. 3c). As predicted, glutamate treatment induced a significant increase in the expression of circFoxO3, which was attenuated by siRNA treatment (Fig. 3d). Those results confirmed that circFoxO3 could affect the cell viability of HT22 cells.

(A)



(B)



(C)

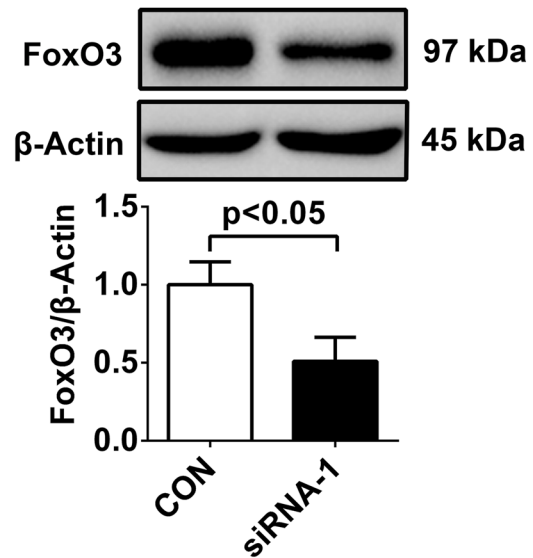


Fig. 2 Efficiency of siRNA-mediated circFoxO3 knockdown in HT22 cells. **a** Sequences of two siRNAs targeting circFoxO3 and representative fluorescence microscopy images delineating transfection

efficiency. Scale bar = 400 μm. **b** Both siRNA-1 and siRNA-2 significantly reduced the expression levels of circFoxO3. **c** siRNA-1 significantly reduced the expression levels of FoxO3 protein in HT22 cells

Blocking circFoxO3 Induces FoxO3 Translocation into the Cytoplasm and Downregulates Bim_{EL} Expression

It was reported that circFoxO3 could regulate the expression of the parent gene FoxO3, thereby regulate the cell cycle and cell survival (Du et al. 2017). We further investigated the effect of circFoxO3 on the FoxO3 protein in HT22 cells. As shown in Fig. 4a, glutamate treatment elevated the protein levels of FoxO3, and silencing of

circFoxO3 partially reduced the protein levels of FoxO3. Furthermore, the subcellular localization of FoxO3 was determined by immunocytochemistry staining. As shown in Fig. 4b, glutamate treatment induced the nuclear localization of FoxO3 in HT22 cells, while circFoxO3 silencing reversed the nuclear translocation of FoxO3 induced by glutamate treatment. The pro-apoptotic protein Bim_{EL} is one of the target genes of FoxO3 (Becker et al. 2004). Therefore, we tested the effect of circFoxO3 on Bim_{EL}.

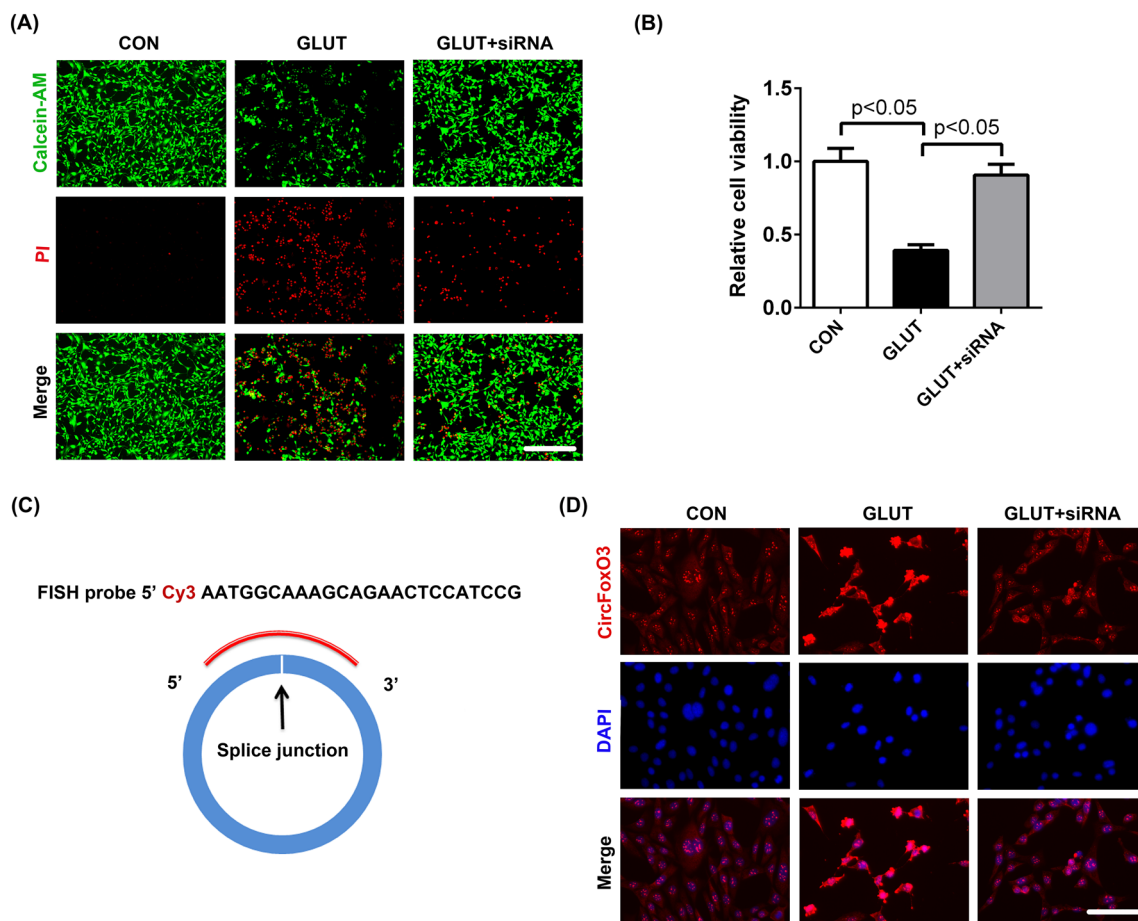


Fig. 3 Blocking circFoxO3 protects HT22 cells against glutamate-induced injury. **a** The Calcein-AM and PI cell viability assay demonstrated that blocking circFoxO3 protects HT22 cells from glutamate-induced injury. Scale bar = 400 μm. **b** Quantitative analysis of data from **a**. **c** Probe sequences used in the binding assay in RNA FISH.

The probe crossed the backsplice junction and was specific for FoxO3 circular RNA. **d** FISH was performed to measure circFoxO3 expression, and it was found that the glutamate-treated HT22 cells had higher levels of circFoxO3 than the controls. However, circFoxO3 siRNA inhibited the expression of circFoxO3. Scale bar = 100 μm

Silencing of circFoxO3 could also decrease the expression of the pro-apoptotic protein Bim_{EL} (Fig. 5).

Silencing of circFoxO3 Reduces the Loss of Mitochondrial Membrane Potential (MMP) Induced by Glutamate

To investigate the effects of circFoxO3 on MMP, JC-1 staining was used to visualize the changes in the MMP of HT22 cells. The mitochondria with normal MMP emitted strong red fluorescence, whereas the mitochondria with decreased MMP emitted green fluorescence. Compared with the control treatment, glutamate treatment resulted in significantly decreased MMP, as evidenced by a significant increase in the green/red fluorescence intensity ratio. siRNA targeting circFoxO3 attenuated this glutamate-induced MMP loss. This group displayed only a slight increase in the green/red fluorescence intensity ratio (Fig. 6).

Silencing of circFoxO3 Reduces the Increase in Glutamate-Induced Oxidative Stress in HT22 Cells

Oxidative stress plays an important role in age-related neurodegenerative diseases. High concentrations of ROS can destroy mitochondrial membranes, and mitochondrial damage leads to further ROS generation (Liu et al. 2019). To examine whether circFoxO3 was involved in the generation of ROS, intracellular ROS levels in HT22 cells were determined using H2DFFDA staining. As shown in Fig. 7, glutamate treatment caused an accumulation of ROS. In contrast, silencing of circFoxO3 attenuated the increase in glutamate-induced ROS in HT22 cells.

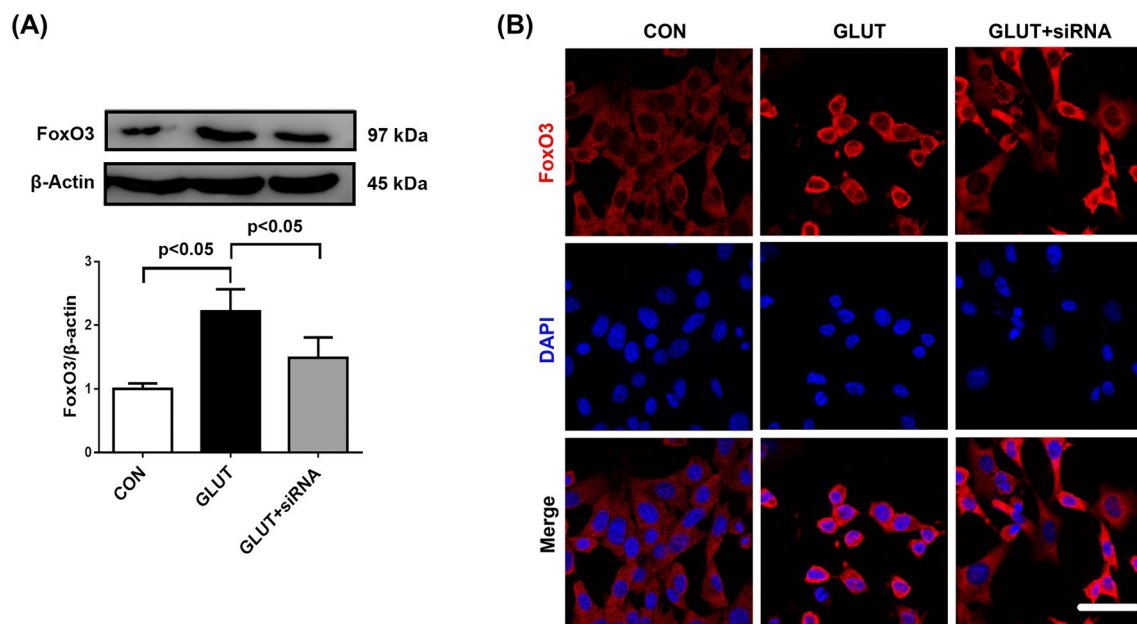


Fig. 4 Blocking circFoxO3 affected the expression level and subcellular location of FoxO3. **a** Western blots demonstrated that glutamate treatment elevated the protein levels of FoxO3, and silencing of circ-FoxO3 partially reduced the protein levels of FoxO3. **b** Glutamate

treatment induced the nuclear localization of FoxO3 in HT22 cells, while silencing of circFoxO3 reversed the nuclear translocation of FoxO3 induced by glutamate treatment. Scale bar = 40 μ m

Silencing of circFoxO3 Protects HT22 Cells from Glutamate-Induced Injury by Regulating the Mitochondrial-Related Apoptosis Pathway

To further investigate whether circFoxO3 was involved in the mitochondrial apoptotic pathway in HT22 cells, Cytochrome *c* and Cleaved caspase-3 were assessed by Western blot analysis. It was found that the expression of Cytochrome *c* and Cleaved caspase-3 was increased in the cells incubated with glutamate. In addition, the inhibition of circFoxO3 abolished the glutamate-induced increase in Cytochrome *c* and Cleaved caspase-3 in HT22 cells (Fig. 8a–d). CircFoxO3 siRNA also reversed the glutamate-induced increase in the activity of Caspase-3 (Fig. 8e), similar to the results obtained using Western blot analysis. TUNEL staining was used to observe the morphology of the apoptotic cells. The TUNEL assay revealed that circFoxO3 siRNA attenuated glutamate-induced cell apoptosis (Fig. 8f). Collectively, the results suggested that circFoxO3 could regulate the Cytochrome *c*/Caspase-3 mitochondrial-related apoptotic pathway in HT22 cells, and the neuroprotective effects of circ-FoxO3 siRNA were mediated through the modulation of Cytochrome *c*/Caspase-3 mitochondrial-related apoptotic signalling (Fig. 9).

Discussion

CircFoxO3 has been reported to be involved in several pathological processes, including tumour progression and cardiomyopathy (Du et al. 2017; Yang et al. 2016). In this study, we first evaluated the expression of circFoxO3 in HT22 neuronal cells and discovered that circFoxO3 is significantly upregulated after glutamate-induced oxidative insult in these cells. Our findings further indicated that circFoxO3 contributes to mitochondrial-mediated apoptosis by activating the FoxO3/Bim_{EL} pathway, which provides a novel direction and target for new treatment strategies for neurodegenerative diseases.

Oxidative stress injury in the central nervous system is critically linked to neurodegenerative diseases (Zeng et al. 2017). With age, the activity of enzymatic antioxidants, i.e., catalase (CAT) and superoxide dismutase (SOD), gradually decreases, resulting in an accumulation of ROS (Gan et al. 2018). Excess ROS cause the damage to mitochondria, which in turn leads to further ROS generation (Ma et al. 2018). We studied whether circFoxO3 is involved in the production of intracellular ROS. We found that glutamate induced an accumulation of intracellular ROS and that circ-FoxO3 silencing reduced the increase in glutamate-induced ROS in HT22 cells. Taken together, the current results

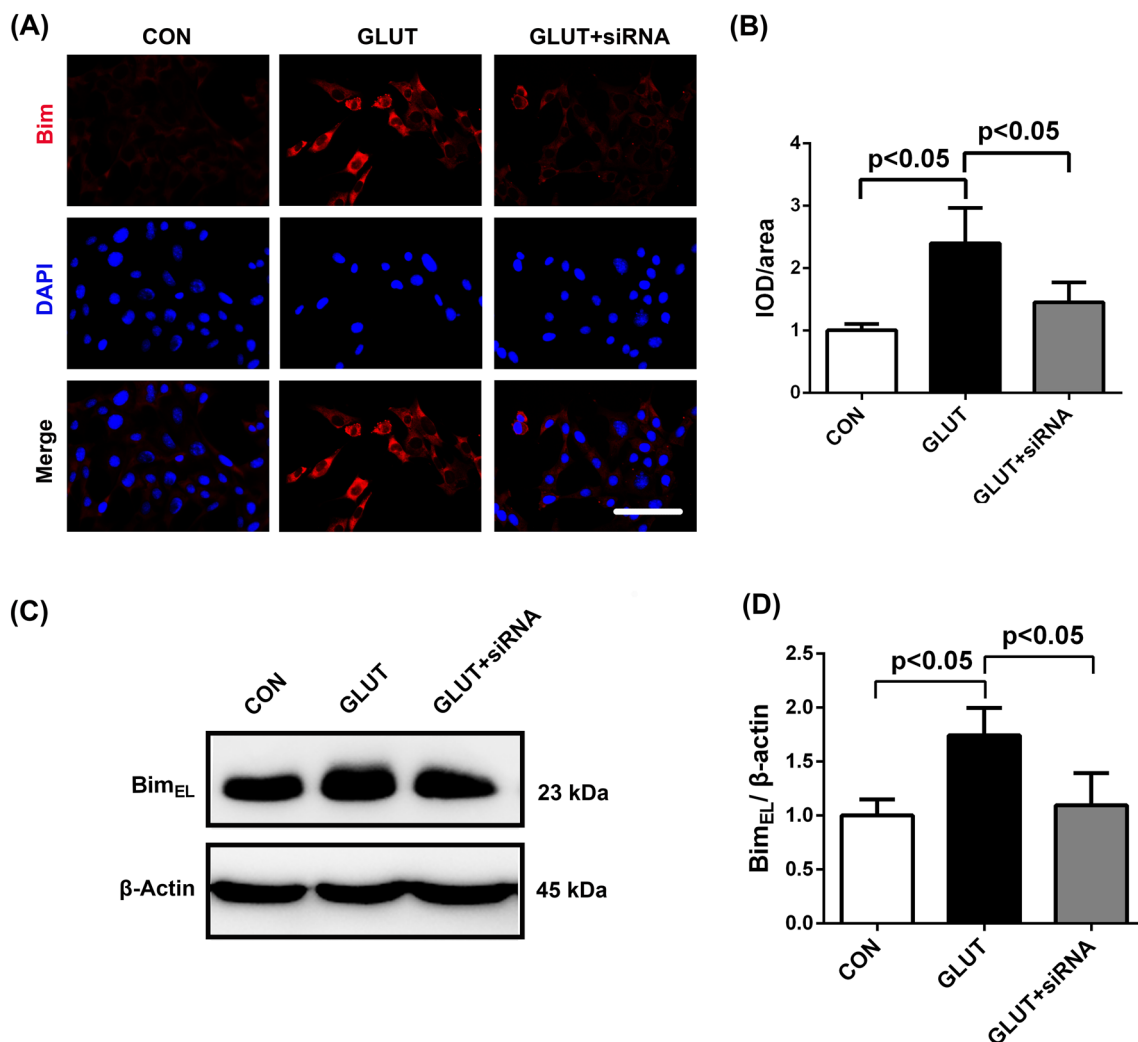


Fig. 5 Blocking circFoxO3 downregulates the expression of Bim. **a** Immunocytochemistry staining demonstrated that glutamate treatment increased the expression levels of Bim, and silencing of circFoxO3 downregulated the levels of Bim. Scale bar = 100 μ m. **b** Quan-

titative analysis of the data from **a**. **c** Western blot demonstrated that glutamate treatment increased the expression levels of the Bim_{EL} protein, and silencing of circFoxO3 downregulated the levels of the Bim_{EL} protein. **d** Quantitative analysis of the data from **c**

provided evidence that ROS production induced by glutamate is associated with circFoxO3. Oxidative stress appears in the early stages of ageing and, hence, is an important therapeutic target (Zeng et al. 2017). Thus, the knockout of endogenous circFoxO3 may prevent oxidative damage and help maintain brain function.

Neuronal apoptosis induced by ROS is one of the important pathological mechanisms of neurodegenerative diseases (Gao et al. 2017). Recently, it was reported that circFoxO3 regulates the tumour growth and myocardial cell survival through the apoptosis pathway (Du et al. 2017; Yang et al. 2016). We hypothesized that circFoxO3 participates in the process of glutamate-induced neuronal cell death by regulating the apoptosis pathway. Our results demonstrated that glutamate treatment increased the activity of Caspase-3, whereas the activity of Caspase-3 was significantly blocked

in circFoxO3 knockdown cells. TUNEL staining was used to observe the morphology of apoptotic cells (Wang et al. 2015). The TUNEL assay revealed that circFoxO3 siRNA could attenuate glutamate-induced cell apoptosis. Our results demonstrate that circFoxO3 participates in the glutamate-induced neuronal apoptosis, which is in accordance with previous studies (Li et al. 2006; Du et al. 2017).

Although previous studies have reported that elevation of circFoxO3 is involved in the apoptotic pathway, its downstream pathway has not yet been elucidated (Du et al. 2017). Our results demonstrate that elevated circFoxO3 levels result in the upregulation and nuclear translocation of FoxO3. The pro-apoptotic gene Bim is a target of FoxO3. The Bim protein exists in three major isoforms, Bim_{EL}, Bim_L and Bim_S, which are generated by alternative splicing. Among those isoforms, Bim_{EL} is the predominant

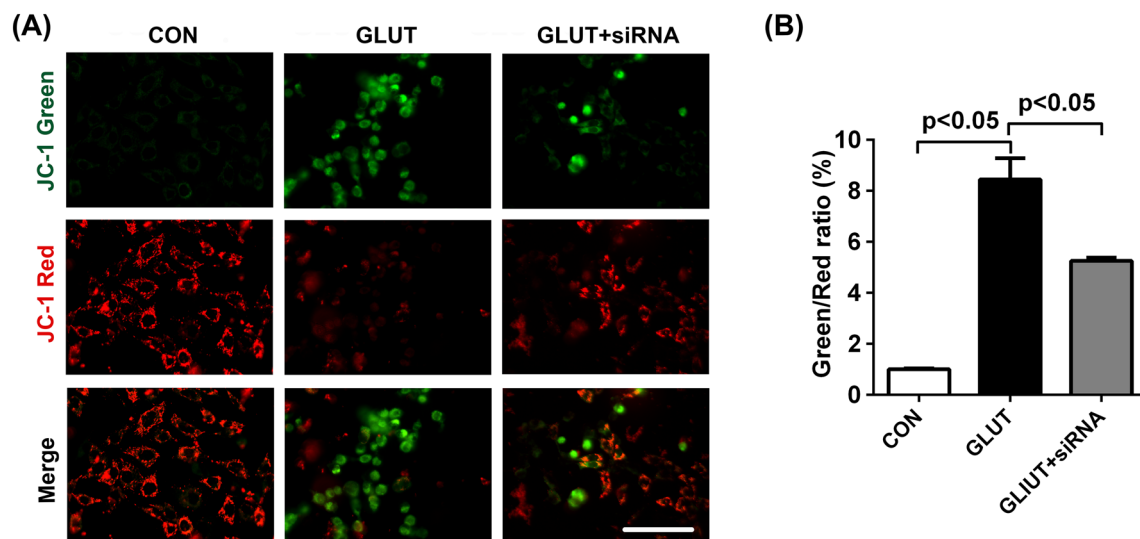


Fig. 6 Silencing of circFoxO3 attenuates glutamate-induced MMP loss in HT22 cells. **a** JC-1 staining was used to measure the MMP. Red fluorescence indicated normal MMP. Green fluorescence indi-

cated damaged mitochondria with loss of MMP. Scale bar = 100 μ m. **b** Quantitative analysis of the ratio of green and red fluorescence intensity

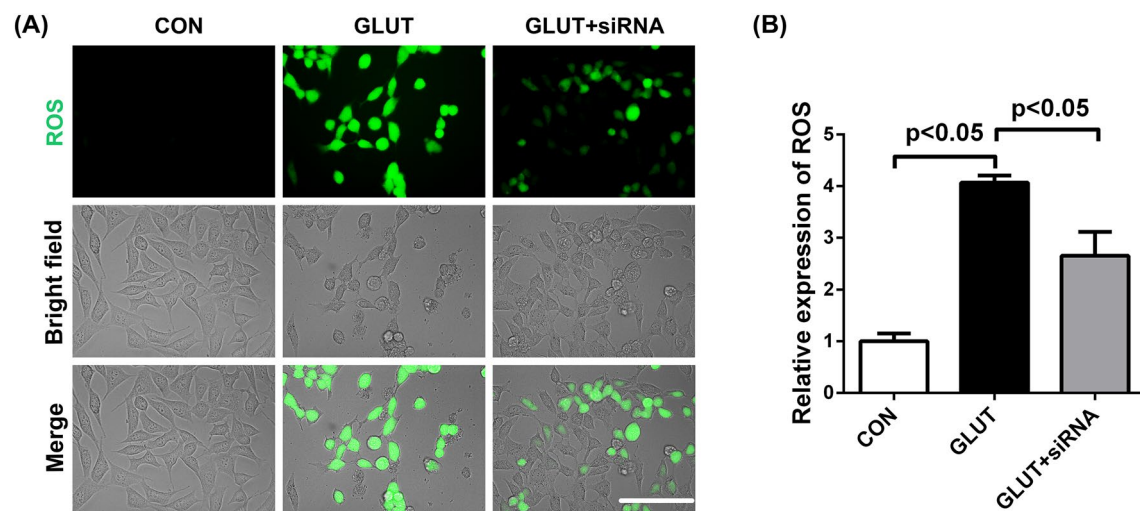


Fig. 7 Silencing of circFoxO3 suppresses the glutamate-induced increase in intracellular ROS. **a** H2DFDA staining showed that circFoxO3 siRNA attenuated the elevation of glutamate-induced ROS. Scale bar = 100 μ m. **b** Quantitative analysis of the data from **a**

isoform and is mainly expressed in neurons (Becker et al. 2004). In the present study, it was found that glutamate treatment could increase the expression of the pro-apoptotic protein Bim_{EL}, whereas silencing of circFoxO3 downregulated the levels of the Bim_{EL} protein. The current results provide evidence that the circFoxO3/FoxO3/Bim_{EL} pathway may be involved in glutamate-induced HT22 cell apoptotic death. As a pro-apoptotic protein, activated Bim will not only activate pro-apoptotic factors (such as Bax and Bak) but also inhibit anti-apoptotic proteins (such as Bcl-2 and Bcl-xL), which promote mitochondrial outer

membrane permeabilization and lead to mitochondrial damage (Guo et al. 2019). To further investigate whether circFoxO3 induces neuronal apoptosis by the mitochondrial apoptosis pathway, the levels of Cytochrome c and Cleaved caspase-3 were assessed by Western blot after glutamate treatment. As expected, significant increases in the expression levels of Cytochrome c and Cleaved caspase-3 were observed in the cells incubated with glutamate. JC-1 staining was used to observe the change in mitochondrial membrane potential. The JC-1 assay revealed that glutamate treatment resulted in a significantly decreased

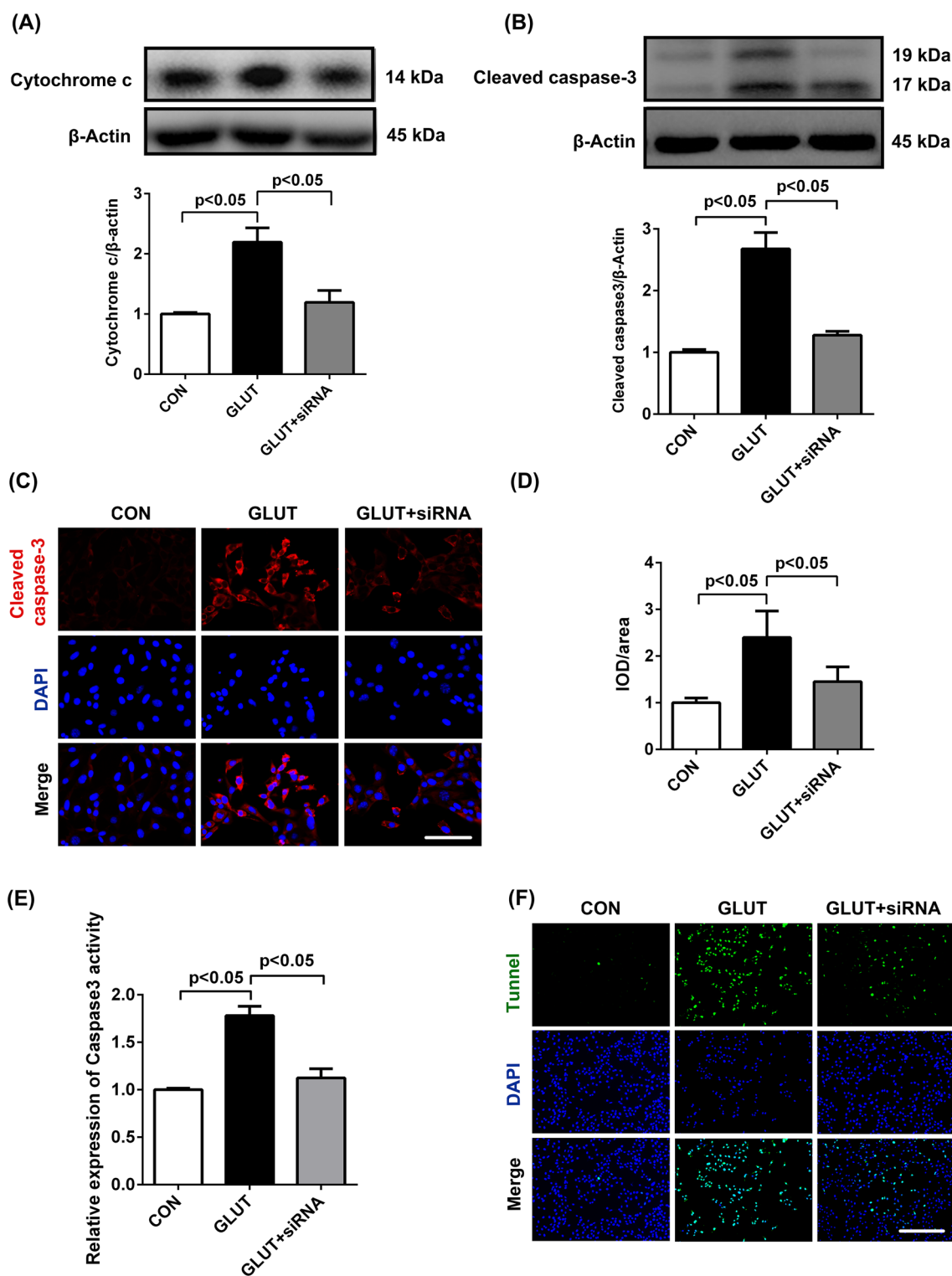


Fig. 8 Blocking circFoxO3 attenuates glutamate-induced mitochondrial apoptosis injury. **a** Western blots demonstrated that the inhibition of circFoxO3 abolished the glutamate-induced increase in Cytochrome c in HT22 cells. **b** Western blots demonstrated that the inhibition of circFoxO3 abolished the glutamate-induced increase in Cleaved caspase-3 in HT22 cells. **c** Immunocytochemistry staining demonstrated that glutamate treatment increased the expression lev-

els of Cleaved caspase-3, and silencing of circFoxO3 downregulated the expression levels of Cleaved caspase-3. Scale bar=100 μ m. **d** Quantitative analysis of the data from **c**. **e** siRNA against circFoxO3 reversed the glutamate-induced increase in the activity of Caspase-3. **f** TUNEL assay revealed that circFoxO3 siRNA significantly attenuated glutamate-induced cell apoptosis. Scale bar=400 μ m

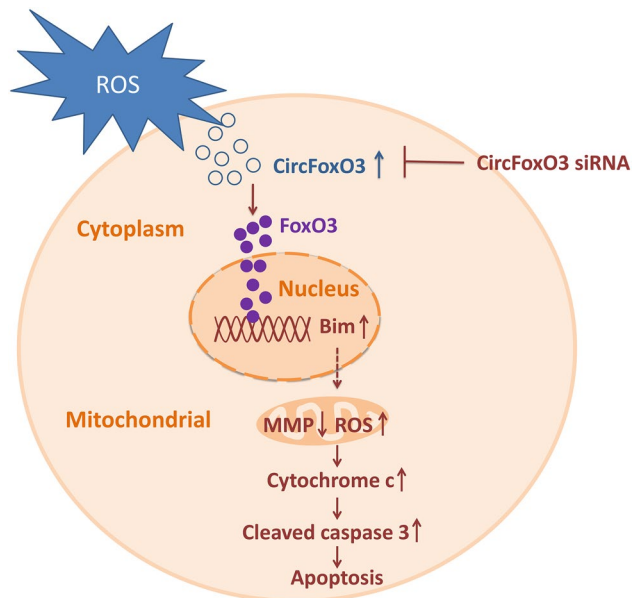


Fig. 9 Hypothetical model of this study. CircFoxO3 is upregulated during oxidative insult, which results in the nuclear translocation of FoxO3 and actually promoted mitochondrial apoptosis in HT22 cells. Blocking circFoxO3 attenuates ROS-induced apoptosis in HT22 cells

MMP. Furthermore, circFoxO3 siRNA attenuated the glutamate-induced increased Cytochrome c and Cleaved caspase-3 and decreased MMP. Taken together, our findings suggested that circFoxO3 resulted in the upregulation of FoxO3/Bim_{EL} and actually promoted mitochondrial apoptosis in HT22 cells.

The data in the present study were based on the HT22 cell line, and there was a lack of primary hippocampus neurons or in vivo assays. This is an important limitation of our study. However, we reported a previously unrecognized role of circFoxO3 in a model of neurodegenerative disease and suggested a potential diagnostic marker and therapeutic target for neurodegenerative diseases. The mechanism of circFoxO3 action requires further investigation. In addition, specific studies are needed to understand whether circFoxO3 has effects on other central nervous system diseases.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

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