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The mechanism of lncRNA TALNEC2 regulating miR-19a-3p/JNK to alleviate cerebral ischemia injury in rats with acute cerebral infarction

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ABSTRACT

Ischemia-reperfusion (I-R) is renowned as a key approach in recovery related to cerebral infarction and further promotes succeeding infarction development. This study investigated the fundamental molecular function of the TALNEC2 in the pathogenesis of cerebral infarction to provide insights into the potential novel therapeutic agents in cerebral infarction. RT-qPCR measured the expression of TALNEC2 and JNK in human neural cell line SH-SY5Y. Cell transfection upregulated or silenced the genes with MTT assay examining cell viability. RT-qPCR detected cell death in the apoptosis biomarker caspase-3, inflammation in the biomarkers C-reactive protein (CRP) and IL-6 and verified cell proliferation via the ki67 and PCNA markers. A luciferase assay was performed to see the luciferase activity and western blotting determined the protein expression of JNK in proliferation and inflammation. The results demonstrated that TALNEC2 was highly expressed after OGD/R treatment in nerve cells after cerebral infarction. In addition, TALNEC2 silencing prevented apoptosis and inflammation of nerve cells after cerebral infarction. TALNEC2 directly interacted with miR-19a-3p to regulate JNK protein expression. Lastly, the miR-19a-3p inhibitor abolished the protective effect of si-TALNEC2 against OGD/R induced damage in vitro. In summary, this study has demonstrated that TALNEC2 is a positive moderator for the pathogenesis of cerebral infarction. Furthermore, our conclusions provide further insights into the interplay among TALNEC2, miR-19a-3p and JNK in cerebral infarction. It has been demonstrated herein that TALNEC2 positively modulates JNK post-transcriptional expression through miR-19a-3p sponging in cerebral Infarction offering a novel therapy target for cerebral infarction.

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Introduction

Ischemia-reperfusion (I-R) is regarded as very vital in the recuperation of ischemic cerebral damage and further retrains succeeding infarction growth (1). Nonetheless, ischemia/reperfusion stimulates apoptosis rate, inflammatory factors, and excess production of responsive oxygen species (ROS), therefore resulting in neuronal damage (2). Nevertheless, this kind of treatment predictably causes ischemia-reperfusion damage and the thrombolytic treatment outcome is inadequate. Collective investigation reports have proven that apoptosis including loss of nerve cells following Ischemia-reperfusion are the leading cause of worsening brain damage (3). And so, it is a great essence to establish innovative treatment techniques to cure cerebral infarction.

In the *homo sapiens'* genetic material, merely 2 percent of the DNA is transcribed into proteins, implying that a large part of the genetic material is left either not translated or frequently translated into noncoding RNAs (4). Long non-coding RNAs (lncRNAs) are elongated than 200 nucleotides originally regarded as not useful chuck, however now many reports their involvement in a diverse array of biological processes such as chromatin regulation, alternative splicing of pre-mRNA, nuclear organization, and human diseases (5). LncRNAs can serve as competing for endogenous RNAs (ceRNAs) to lessen the expression of microRNAs (miRNAs), leading to miRNA function suppression in cells (6). For example, down-regulation of lncRNA SNHG1 reduced $A\beta_{25-35}$-induced neuronal damage by modulating KREMEN1 via serving as a ceRNA of miR-137 in neuronal cells (7). In addition, long non-coding RNA SNHG1 served as a competitive endogenous RNA to moderate PDCD4 expression via sponging miR-195-5p in hepatocellular carcinoma (8). These outcomes impelled the authors to investigate the fundamental mechanistic functions of TALNEC2 in Cerebral Infarction.

A study has found a miR-19a-3p role in upregulation in rat I/R brain tissues (OGD/R) induced SH-SY5Y cells, and the miR-19a-3p inhibitor protects against cerebral I/R injury. It is shown that miR-19a-3p binds to the 3′UTR region of IGFBP3 mRNA causing the increased expression of IGFBP3 in OGD/R induced SH-SY5Y cells. TALNEC2 was highly expressed in OGD/R induced SH-SY5Y cells in glioma cells and glioma stem cells (GSCs). The expression of TALNEC2 is associated with the increased tumorigenic potential of GSCs and hence TALNEC2 is an attractive therapeutic target.

In vivo, miR-19a-3p promoted liver tumor growth, and AKT is a likely target gene of miR-19a-3p. The study also states that knockdown of TALNEC2 reverses the brain injury and neurological deficits induced by I/R in vivo, and elevated miR-19a-3p mediates cerebral ischemic injury thus might offering hope of a novel therapeutic target for

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ischemic stroke injury treatment

In the present investigation, we utilized the SH-SY5Y cell oxygen-glucose deprivation and re-oxygenation (OGD/R) model to ascertain whether TALNEC2 expression was transformed in cerebral infarction. Mainly our study investigated the fundamental molecular function of the TALNEC2JNKLncRNA TALNEC2 in the pathogenesis of cerebral infarction to provide insights into the potential novel therapeutic agents in cerebral infarction.

Materials and Methods

Cell culture preparation

The cell culture and transfection were performed as described below. The human neural cell line SH-SY5Y utilized in this study was obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China), and routinely grown in Dulbecco's Modified Eagle medium (DMEM; Gibco, Grand Island, USA) or RPMI-1640 (Gibco) with the addition of 1% penicillin/streptomycin (Gibco). Then, after 48hrs, the substrate was superseded with 10% fetal bovine serum (FBS; HyClone, Logan, USA) coupled with 5 μM RA aimed at neuronal segregation. The cells were sustained in a humidified atmosphere at 37˚C with 5 percent CO2 in an incubator and were grown to 85%–95% confluency before use.

The Oxygen-Glucose Deprivation-Reoxygenation model

To mimic ischemic-resembling conditions in vitro, SH-SY5Y neural cell line cultures were subjected to oxygen-glucose deprivation (OGD/R) for varying periods prior to experimenting and followed the method described in Ref (9).

Cell transfection

To study the functional roles of the lncRNA's in OGD/R-exposed SH-SY5Y cells, miR-19a-3pmimics, inhibitors, negative control miR-19a-3p, TALNEC2 siRNA, JNK siRNAs, or negative siRNA, or two of these treatments were transfected into SH-SY5Y cells at operating dilutions 1:1 (v/v) utilizing Lipofectamine™ 3000 Transfection reagent (Beyotime, Shanghai, China) following manufacturer's guidelines. Following a 6 h transfection observation, SH-SY5Y cells were challenged to OGD/R/R preservation of (6-hour OGD/R and 24-hour reoxygenation). The transfection efficacies of the miR-19a-3por siRNA in SH-SY5Y cells were validated via RT-qPCR to measure miR-19a-3por JNK expression. The transfection efficacies of the JNK siRNA were validated via Western blotting evaluation.

The over-expressed TALNEC2 plasmids and respective negative control plasmids were obtained from Guangzhou Fulengen Co. Ltd. Cell transfection of cultured SH-SY5Y neural cell line was done using Lipofectamine 6000 (Beyotime, Shanghai, China) to transfect oe-TALNEC2 plasmids or negative control plasmids following the manufacturer's guidelines and transfection efficiency was analyzed after 48 hrs.

Real Time-quantitative PCR (RT-qPCR) assay

Entire RNA was separated from cell-free fractions of SH-SY5Y neural cell line using Beyozol mixture (#R0011, Beyotime, Shanghai, China) then reverse transcription of1 μg RNA was done for each specimen to cDNA using BeyoRT™ cDNA First Chain Synthesis Kit (#D7166, Beyotime, Shanghai, China) following guidelines provided by the manufacturer. Quantitative Real Time-PCR (qRT-PCR) assays were conducted using Beyofast™ SYBR Green QPCR Mix (Beyotime, China) and an associated JNK qRT-PCR detection kit (Beyotime, China) to measure quantities of lncRNA, miR-19a-3pand JNK expression, respectively on an Applied Biosystems Vii7 RT-qPCR instrument (ABI, Vernon, CA, USA) with. GAPDH was used as the internal control and the $2^{-\Delta\Delta CT}$ method.

Flow cytometry apoptosis assay

Flow cytometry assay was performed to examine the apoptosis rate. The human neural cell line SH-SY5Y exposed to OGD/R/R at varied time periods were transitorily transfected with miR-19a-3pmimics, inhibitors, negative control miR-19a-3p, TALNEC2 siRNA, JNK siRNAs, or negative siRNA. Then, the treated human neural cells were nurtured by using annexin V/PI solution (Jiamay Biotechnology, Guangzhou, Guangdong, China) for 30 minutes at room temperature, in darkness. Then, the apoptosis rate was measured by the CyFlow Counter flow cytometer (Morey Biosciences, Xuhui, Shanghai, China).

Methyl thiazolyl tetrazolium (MTT) assay

The MTT assay was performed 48 hours after transfection in which SH-SY5Y cells exposed to OGD/R/R at varied time periods were transitorily transfected with miR-19a-3pmimics, inhibitors, negative control miR-19a-3p, TALNEC2 siRNA, JNK siRNAs, or negative siRNA were counted and seeded in 4x 96-well plates (2 × 102, 200 μL per well, in 8 replicated wells). Methyl thiazolyl tetrazolium solution of 20 μL was supplemented onto each well for incubation for 3.5 hours at 37°C, then, incubation was stopped, and culture tissue was extracted. 150 μL dimethyl sulfoxide (DMSO) (Sigma, Englewood Cliffs, NJ, USA) was supplemented into each corresponding MTT-treated well to dissolve the minerals and gently stirred for 10 minutes in an enzyme-linked immunosorbent sensor. The wavelength was measured at an absorbance of 490 nm at 0h, 24h, 48h and 72h, respectively by using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to detect cell viability.

Western blotting assay

Western blot was performed to evaluate the expression level of the proteins of cell proliferation, inflammation, and apoptosis-related biomarkers, including ki67, PCNA, C-reactive protein (CRP), IL-6, caspase-8 and caspase-3. The SH-SY5Y neural stem cell line cultures were subjected to oxygen-glucose deprivation and reoxygenation (OGD/R) for varying periods and then the proteins were extracted and washed twice with cold PBS and lysed in sample loading buffer. The expression of the load-up buffer was 1.5 percent SDS, 10 percent glycerol, 5 mM β-mercaptoethanol, bromophenol blue and 75 mM Tris (pH 7.0). Entire cell lysates were isolated by SDS-PAGE of 12 percent gel and the proteins were transferred onto a polyvinylidene fluoride film. Additionally, the films were incubated and searched with the following antibodies: ki67, PCNA, C-reactive protein (CRP), IL-6, caspase-8 and caspase-3 enlisted in Table 1 under the temperature of 4°C overnight. The immunoblots were obtained and seen

Table 1. Antibodies.

Protein Examined	Primary Antibody	Secondary Antibody
PCNA	Rabbit Polyclonal, (PA5-27214) (ThermoFisher, Scientific, NY, US) diluted at 1:1000.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555 (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
Ki-67	Rabbit polyclonal to Ki67 (ab15580), (Abcam, Cambridge, US) diluted at 1:900.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555, (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
caspas-3	Rabbit polyclonal to Caspase-3 (ab13847) (Abcam, Cambridge, UK) diluted at 1:1000.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555, (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
caspas-8	Rabbit polyclonal to Caspase-8 (ab25901), Abcam, Cambridge, UK) diluted at 1:1000.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555, (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
CRP	Rabbit Polyclonal (PA5-79070), (ThermoFisher, Scientific, NY, US) diluted at 1:1000.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555, (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
IL-6	Rabbit Polyclonal (ARC0062), (ThermoFisher, Scientific, NY, US) diluted at 1:1000.	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 555, (ThermoFisher, Scientific, NY, US) diluted at 1:1000.
GAPDH	Mouse monoclonal (6C5) to GAPDH - Loading Control, (ab8245, Abcam, Cambridge, UK) dilution rate 1:2000.	Rabbit monoclonal (R18-2) Anti-Rat IgG Fc (ab125900, Abcam, Cambridge, UK) dilution rate 1:1000.

by ECL Western blot substrate (Thermo Fisher Scientific). GAPDH was used as an internal control. The analysis of each group was repeated in three-fold. The image J detection system was employed to determine the concentration of the bands.

Bioinformatics analysis

The target putative binding sites for TALNEC2 and miR-19a-3p were predicted using Starbase.

Luciferase assay

The target sequence miR-19a-3pwith the wild type (WT) or mutant type (MT) TALNEC2 binding locations were synthesized and replicated onto a pGL3 Dual-luciferase Target Vector (Promega, Madison, WI, USA), to establish Wild Type and Mutant Type TALNEC2 plasmids. These Wild Type or Mutant Type TALNEC2 plasmids were co-transfected into SH-SY5Y neural stem cell line with NC plasmids or miR-19a-3pmimic (Sigma-Aldrich; Merck KGaA) using Lipofectamine 2000 following manufacturer's instructions. At 48h time interval, luciferase assay was performed with the Dual-Luciferase Reporter Assay method (Promega), following protocol specified by the manufacturer. The firefly luciferase activities were normalized against Renilla luciferase activity.

Statistical analysis

The experiments were conducted separately three times and experimental information has been shown in form of mean and standard error (SE). The statistical evaluation was performed by GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 18.0 version (SPSS Inc., Chicago, IL, USA). Student's t-test, and ANOVA analysis was applied. The level of significance was $P<0.05$ to show a statistically significant difference.

Results and discussion

TALNEC2 was highly expressed following OGD/R treatment in nerve cells after cerebral infarction

To investigate whether TALNEC2 expression was modified in nerve cells, TALNEC2 expression was instantly measured after 6 hours of Oxygen-Glucose-Deprivation and through various re-oxygenation periods by employing RT-qPCR. Remarkably high levels of TALNEC2 were noticed in the SH-SY5Y cell exposed to the OGD/R treatment model at varying periods and highest being observed at the 48-hour mark in contrast to the normal control group (Fig.1A). This trend was confirmed by RT-qPCR assay on pro-proliferation markers Ki67 and PCNA in which the up-regulated TALNEC2 expression levels corresponded to increased Ki67 and PCNA levels compared with control treatment (Fig. 1B). The upregulated TALNEC2 levels were found to dramatically increase cell viability at varying periods and highest being observed at the 48-hour mark after the MTT assay was performed under the OGD/R treatment model contrasting to the control treatment (Fig.1C).

The caspase-8 and caspase-3 activity verified the apoptosis rate in the SH-SY5Y cell exposed to OGD/R treatment. Similarly, increased caspase-8 and caspase-3 was observed in the OGD/R treatment coupled with overexpressed TALNEC2 expression at varying time periods and highest being observed at the 48-hour mark (Fig.1D).

The results for CRP and IL-6 biomarkers demonstrated that inflammation remarkably increased with time (0h-48h) in the OGD/R SH-SY5Y exposed cell line in contrast to the unexposed cell line (Fig. 1E). But the increase was incremental with time with higher values for both CRP and IL-6 being noticed after 48 hours. Then, the FCM assay verified apoptosis rate which was remarkably increased corresponding to OGD/R treatment overexpressed TALNEC2 expression compared with normal treatment (Fig.1F). In

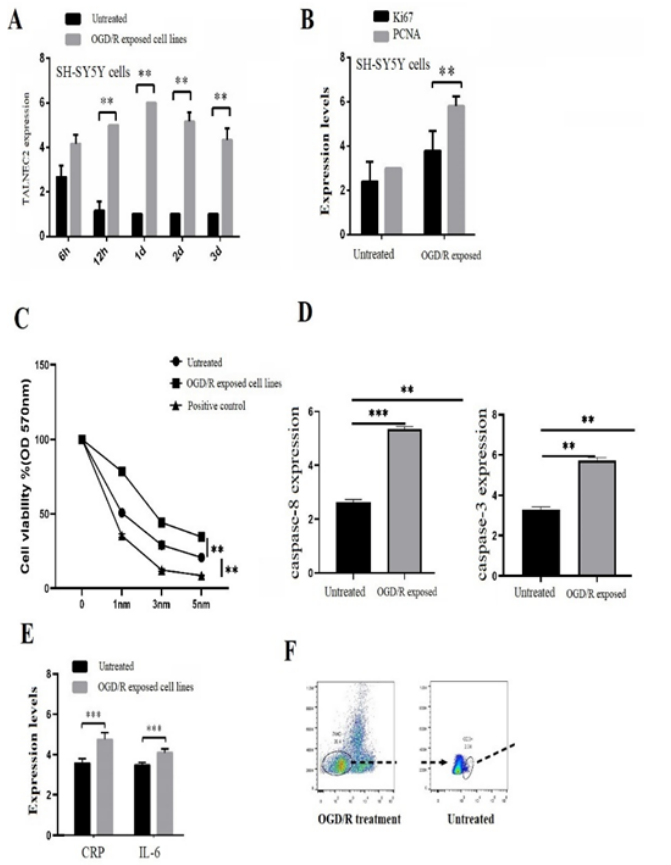

Figure 1. TALNEC2 was highly expressed following OGD/R treatment in nerve cells after cerebral infarction. A) RT-qPCR examined TALNEC2 expression in human neural cell line SH-SY5Y (untreated and OGD/R exposed cell lines) at varying periods ($p<0.05$). B) RT-qPCR assay examined cellular proliferation in pro-proliferation markers (Ki67 and PCNA) based on TALNEC2 expression in human neural cell line SH-SY5Y (untreated and OGD/R exposed cell lines). C) MTT assay examined cellular viability based on TALNEC2 expression in human neural cell line SH-SY5Y untreated and OGD/R exposed cell lines). D) RT-qPCR examined the expression of apoptosis biomarkers caspase-8 and caspase-3 in human neural cell line SH-SY5Y (untreated and OGD/R exposed cell lines) at varying periods ($p<0.05$) E) RT-qPCR examined the expression of inflammation biomarkers CRP and IL-6 in human neural cell line SH-SY5Y (untreated and OGD/R exposed cell lines) at varying periods ($p<0.05$). F) FCM Apoptosis assay examined cellular apoptosis rate based on si-TALNEC2 expression in human neural cell line SH-SY5Y (OGD/R exposed cell lines) at varying periods ($p<0.05$).

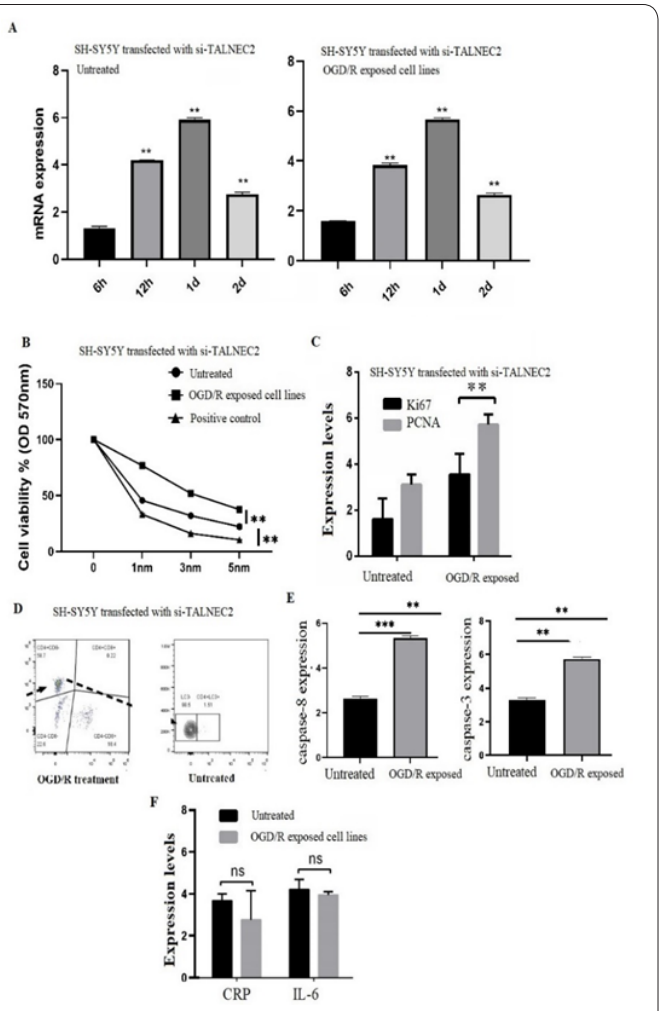

Figure 2. Silenced TALNEC2 suppresses apoptosis and inflammation of nerve cells after cerebral infarction. A) RT-qPCR examined TALNEC2 knockdown efficacy at different periods OGD/R treated human neural cell line SH-SY5Y. B-1) MTT assay examined cellular viability based on si-TALNEC2 expression in human neural cell line SH-SY5Y in OGD/R exposed cell lines ($p<0.05$). B-2) RT-qPCR assay examined cellular proliferation in pro-proliferation markers (Ki67 and PCNA) based on si-TALNEC2 expression in human neural cell line SH-SY5Y (OGD/R exposed cell lines) ($p<0.05$). C-1) FCM Apoptosis assay examined cellular apoptosis rate based on si-TALNEC2 expression in human neural cell line SH-SY5Y (OGD/R exposed cell lines) at varying periods ($p<0.05$). C-2) RT-qPCR examined the expression of apoptosis biomarkers caspase-8 and caspase-3 based on si-TALNEC2 expression in human neural cell line SH-SY5Y (OGD/R exposed cell lines) at varying periods ($p<0.05$) D) RT-qPCR examined expression of inflammation biomarkers CRP and IL-6 in human neural cell line SH-SY5Y (untreated and OGD/R exposed cell lines) at varying periods ($p<0.05$).

general, these outcomes implied that the overexpression of lncRNA TALNEC2 promoted cerebral infarction injury.

TALNEC2 knockdown prevents apoptosis and inflammation of nerve cells after cerebral infarction

The human neural stem cell line SH-SY5Y exposed to OGD/R at varying times (12h-48) was transfected with either negative control or si-TALNEC2 to understand the effect of TALNEC2 on inflammation, cell growth ability and cell death at varying times (12h-48). Firstly, RT-qPCR was conducted to examine transfection efficacy and the results demonstrated a remarkable decrease in TALNEC2 expression for si-TALNEC2 transfected cells compared to the negative control group in each varying time (12h-48) (Fig. 2A, $p<0.05$). This was followed by analyzing the effect of TALNEC2 on cellular viability in SH-SY5Y cells examined by MTT assay. The results demonstrated decreased cell viability with time for si-TALNEC2 transfected cells contrasting the negative control group in SH-SY5Y subjected to OGD/R with observations made after 12h, 24h and 48h had elapsed (Fig. 2B, $p<0.05$). And the inhibitory effect on cell viability improved with incremental time periods. Then, results by RT-qPCR assay in pro-proliferation markers (Ki67 and PCNA) confirmed a dramatically decreased level of Ki67 and PNCA with time for si-TALNEC2 transfected cells contrasting the negative control group in SH-SY5Y cells exposed to OGD/R conditions (Fig. 2C, $p<0.05$). The results after apoptosis were performed indicated a significantly decreased in apoptosis rate for si-TALNEC2 transfected cells contrasting the

negative control group in SH-SY5Y subjected to OGD/R with observations made after 12h, 24h and 48h had elapsed (Fig. 2D, p<0.05). And the decreased effect on apoptosis improved with incremental periods. Similarly, decreased caspase-8 and caspase-3 activity was observed in si-TALNEC2 treated cells with the OGD/R model at varying periods and lowest being observed at the 48-hour mark compared with control treatment (Fig. 2E, p<0.05). The inflammation was verified by RT-qPCR which examined the expression of inflammation biomarkers CRP and IL-6 in human neural cell line SH-SY5Y exposed to OGD/R conditions with observations made after 12h, 24h and 4h had elapsed (Fig. 2D, p<0.05). The outcomes showcased a dramatic significant decrease in CRP and IL-6 activity with time increment in si-TALNEC2 treated cells with the OGD/R model at varying periods and lowest being observed at the 48-hour mark compared to control treatment. The results suggest that silenced TALNEC2 suppresses apoptosis and inflammation of nerve cells after cerebral infarction.

TALNEC2 directly interacts with miR-19a-3p to regulate JNK protein expression

The bioinformatics analysis tool (Starbase) was applied to obtain the putative binding sites between miR-19a-3p and TALNEC2. The results revealed the predicted binding locations among miR-19a-3p and TALNEC2-3' UTR (Fig. 3A). Thereafter, a luciferase reporter vector comprised of Wild Type or Mutant Type TALNEC2 bonded sites were constructed to verify the connection. Subsequently, negative control or miR-19a-3p mimics were transfected with TALNEC2 Wild Type or Mutant Type to verify the luciferase activity of human neural cell line SH-SY5Y. The luciferase activity results showed reduced luciferase activity of WT-TALNEC2 for miR-19a-3p mimics transfected cell line contrasting to the negative control. However, no effect was observed in the luciferase activity of MT-TALNEC2 (Fig. 3B, P<0.05). The expression level of miR-19a-3p was examined by RT-qPCR in the SH-SY5Y cell line after silencing TALNEC2. The results as determined by Real Time-qPCR demonstrated that the expression level of miR-19a-3p was dramatically increased in si-TALNEC2 compared with the negative control (Fig. 3C, P<0.05). The interaction of JNK and miR-19a-3p mimic expression was detected by RT-qPCR assay that determined mRNA protein level after inhibiting miR-19a-3p expression. The results indicated remarkably low JNK expression in the miR-19a-3p expression group and dramatically increased JNK expression in the miR-19a-3p inhibitor group in contrast to the negative control respectively (Fig. 3D, P<0.05).

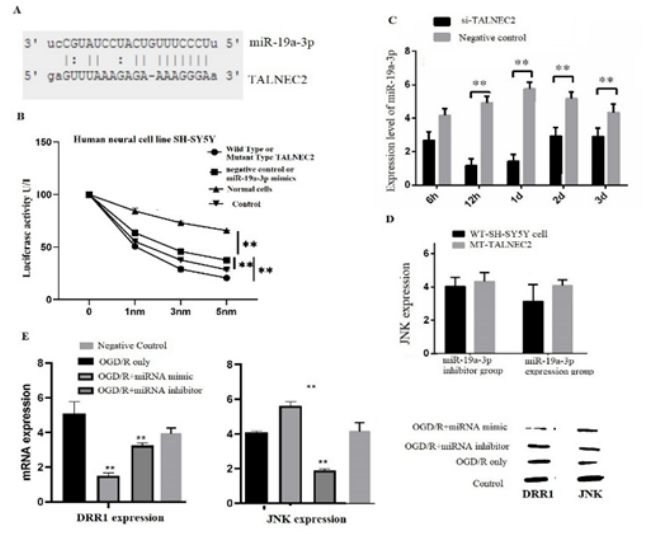

Figure 3. TALNEC2 directly interacts with miR-19a-3p to regulate JNK protein expression A) Bioinformatics analysis tools (Starbase) produced target putative binding sites for TALNEC2 and miR-19a-3p. B) Dual-luciferase reporter verified the actual binding position in WT and MUT TALNEC2 luciferase activity after transfection with miR-19a-3p mimics. C) RT-qPCR examined miR-19a-3p expression with si-TALNEC2 expression. D) RT-qPCR examined JNK expression with miR-19a-3p interaction in human neural cell line SH-SY5Y (p<0.05). E) RT-qPCR examined JNK expression with miR-19a-3p interaction in human neural cell line SH-SY5Y treated with OGD/R (p<0.05).

In addition, the SH-SY5Y cells were treated with OGD/R challenge and transfected to either Negative Control, OGD/R only, OGD/R+miRNA mimic or OGD/R+miRNA inhibitor. Notably, the DRR1 expression was the lowest OGD/R+miRNA mimic group compared with the rest of the groups and JNK was remarkably highest in the OGD/R+miRNA inhibitor contrasting to the rest of the groups (Fig. 3E, P<0.05). These results suggested that TALNEC2 directly interacts with miR-19a-3p to regulate JNK protein expression.

MiR-19a-3p inhibitor abolishes the protective effect of si-TALNEC2 against OGD/R-induced injury in vitro

It has already been verified that miR-19a-3p was found to be regulated and targeted by TALNEC2 human neural stem cell line SH-SY5Y. Therefore, we explored further to understand the underlying molecular mechanisms between miR-19a-3p and si-TALNEC2 when exposed to OGD/R by conducting functional experiments. Firstly, the cells were treated to OGD/R challenge followed by transfection Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA. This was followed by an MTT assay to examine cellular viability. The results produced remarkably highest cell viability in the OGD/R+miRNA inhibitor and OGD/R+miRNA inhibitor groups respectively compared to the negative control, OGD/R only, and OGD/R+si-lncRNA group (Fig. 4A, P<0.05). In addition, pro-proliferation markers exhibited similar trends with the highest Ki67 and PCNA levels observed in OGD/R+miRNA inhibitor and OGD/R+miRNA inhibitor groups respectively compared to the negative control, OGD/R only, and OGD/R+si-lncRNA groups (Fig. 4B, P<0.05). Then, the FCM assay examined the apoptotic activity in the cells in which the apoptosis rates were significantly increased in OGD/R+miRNA inhibitor and OGD/R+miRNA inhibitor groups respectively compared to the negative control, OGD/R only, and OGD/R+si-lncRNA groups. This was verified by the caspase-8 and caspase-3 activity, the pro-apoptosis markers. The outcomes verified dramatically increased caspase-8 and caspase-3 activity in OGD/R+miRNA inhibitor and OGD/R+miRNA inhibitor groups respectively compared to the negative control, OGD/R only, and OGD/R+si-lncRNA groups (Fig. 4C, P<0.05).

Even though there is a large body of evidence that validates ectopic TALNEC2 expression in numerous cancers (10), its expression and biological functions in cerebral infarction still are yet to be described since they are elusive. Our study verified remarkably high TALNEC2 expression

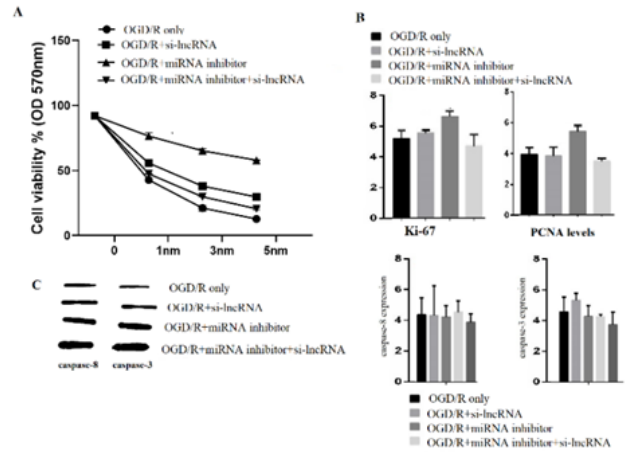

Figure 4. MiR-19a-3p inhibitor abolishes the protective effect of si-TALNEC2 against OGD/R-induced injury in vitro. A) MTT assay examined cellular viability in co-transfected cells with Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA ($p<0.05$). B) RT-qPCR examined the expression of pro-proliferation markers (Ki67 and PCNA) in Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA ($P<0.05$). C) FCM apoptosis assay apoptosis rate in co-transfected cells with Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA ($p<0.05$). D) RT-qPCR examined the relative expression of cell death biomarkers (caspase-8 and caspase-3) in co-transfected cells with Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA ($p<0.05$). E) RT-qPCR examined the relative expression of inflammation markers (CRP and IL-6) in co-transfected cells with Negative Control, OGD/R only, OGD/R+si-lncRNA, OGD/R+miRNA inhibitor, or OGD/R+miRNA inhibitor+si-lncRNA ($p<0.05$).

following treatment with OGD/R conditions in contrast to normal treatment groups.

This significantly upregulated expression affected cell viability, apoptosis, and inflammation by means of dramatically elevating cell viability, apoptosis rate and inflammation via pro-inflammation markers which promoted cerebral infarction progression. Additionally, small interfering RNAs-mediated down-regulation of TALNEC2 dramatically decreased cell viability, apoptosis rate and inflammation in nerve cells exposed to the OGD/R test. Collectively, these data back up the notion that TALNEC2 is very well correlated to cerebral infarction advancement.

A new-fangled mechanistic role of lncRNA TALNEC2 was presented which has been proposed in LncRNA SNHG1 played a protective role to neuronal apoptosis in cerebral infarction rats viaPI3K/Akt network (11) and not long ago, Yang and Zi (12) showcased that TALNEC2 relieves OGD provoked damage in Brain microvascular endothelial cell (BMEC) through miR-338/HIF-1α axis. Additionally, the Long Non-Coding RNA SNHG1 was also found to reduce cell Apoptosis via modulating miR-195 and BCL2-Like Protein 2 in Human Cardiomyocytes (13). By scanning through these investigations, an all-encompassing understanding of the fundamental molecular mechanistic function of more lncRNAs could advance the establishment of novel therapeutic approaches related to cerebral infarction.

Discoidin domain receptor tyrosine kinase 1 (JNK) is involved modulation of cell growth, differentiation and metabolism and its expression is mainly restricted to epithelial cells including the brain. Growing evidence points out that JNK acts an essential function in cerebral infarction. For instance, miRNA-199a-5p safeguarded against cerebral infarction damage through JNK silencing in Rats (14). Reduced miR-199a-5p expression causes elevated cell invasion via functional dysregulation of JNK activity in hepatocellular carcinoma (15). MiR-199a-5p deficiency overexpressed JNK exacerbated colorectal cancer via epithelial-to-mesenchymal transition associated network activation (16). MiR-199a/b-5p suppressed Lymphangiogenesis via targeting JNK in Corneal Injury (15). Thus, it was hypothesized that TALNEC2 puts forth its functional role by means of modulating JNK.

The mechanistic activity of lncRNAs comprises transcriptional and post-transcriptional modulation. Recently, other investigators have uncovered a novel controlling mechanism involving lncRNAs as competitive endogenous RNAs that sponge microRNAs, consequently taking part in post-transcriptional processing (17). The ceRNA role by TALNEC2 on miR-19a-3p was verified by the information presented in this study which was initially predicted via bioinformatics analysis to ascertain TALNEC2 and miR-19a-3p interplay which was indeed confirmed that TALNEC2 and miR-19a-3p have binding sites. Then, silenced TALNEC2 expression resulted in remarkable increased miR-19a-3p expression implying that TALNEC2 adversely modulates miR-19a-3p expression. However, knocking down of TALNEC2 moderation of miR-15-5p level effectively overturned the protective influence embodied by TALNEC2 siRNA. In addition, JNK expression increased following miR-19a-3p inhibition. These results verified that TALNEC2stimulates cell injury via modulating miR-19a-3p. Therefore, following this verification of the association among TALNEC2, miR-19a-3p and JNK after cerebral infarction injury, the downstream target for miR-19a-3p was identified in this interaction.

It has been demonstrated that miR-19a-3p upregulation by means of miR-19a-3p mimics dramatically reduced JNK buildup while silencing miR-19a-3p expression by means of miR-19a-3p inhibitor remarkably elevated JNK levels. Additionally, the miR-15-5p mimic overturned JNK accumulation existence of the OGD/R challenge. These evaluations demonstrated that TALNEC2 sponged miR-19a-3p to provoke cell injury via JNK upregulation. In fact, preceding investigations have demonstrated that genes involved with protein transcription may perhaps be modulated through numerous non-protein transcriptional RNAs whereas a single non-protein transcriptional RNA may as well moderate several genes associated with protein transcription (18). In this manner, whether JNK could be modulated by additional sponging lncRNAs, and whether TALNEC2 could serve the role of sponge lncRNA to modify some other crucial moderator expressions in cerebral infarction, still necessitates further research. The data herein does not consolidate the functional role ofTALNEC2 and JNK as pro-apoptosis and pro-inflammatory genes while miR-19a-3p as an anti-apoptosis and anti-inflammatory gene in cerebral infarction injury advancement, but also discloses a novel fundamental molecular mechanism in which a TALNEC2 stimulates cell injury via modulating miR-19a-3p and JNK expression.

In summary, this current study has documented TALNEC2 as a positive moderator for the pathogenesis

of cerebral infarction. Furthermore, our conclusions provide further insights into the interplay among TALNEC2, miR-19a-3p and JNK in cerebral infarction. It has been demonstrated herein that TALNEC2 positively modulates JNK post-transcriptional expression through miR-19a-3p sponging in cerebral Infarction offering a novel therapy target for cerebral infarction.

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Authors' contribution
This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

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