LncRNA ANRIL protects against oxygen and glucose deprivation (OGD)-induced injury in PC-12 cells: potential role in ischaemic stroke

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ABSTRACT
LncRNA ANRIL was reported to be closely related to ischaemic stroke (IS). In this study, we used oxygen-glucose deprivation (OGD) to stimulate rat adrenal medulla-derived pheochromocytoma cell line PC-12 to construct an in vitro IS cell model and investigated the role of ANRIL and the underlying mechanism. PC-12 cells were stimulated by OGD and/or transfected with pc-ANRIL, si-ANRIL, miR-127 mimic, miR-127 inhibitor, pEX-Mcl-1, sh-Mcl-1 and their negative controls. Cell viability, apoptosis, mRNA and protein expression was detected using CCK-8 assay, flow cytometry assay, qRT-PCR and western blot, respectively. Results showed that OGD-induced PC-12 cell injury and decreased ANRIL expression. ANRIL overexpression significantly reduced OGD-induced PC-12 cell injury evidenced by increasing cell viability and decreasing apoptosis, while ANRIL silence led to the opposite results. Meanwhile, dysregulation of ANRIL altered the expression of apoptotic proteins. Furthermore, ANRIL negatively regulated miR-127 expression. miR-127 overexpression significantly enhanced OGD-induced PC-12 cell injury. In addition, Mcl-1 expression was negatively regulated by miR-127, besides ANRIL up-regulated Mcl-1 expression by down-regulation of miR-127. Mcl-1 overexpression alleviated cell injury and miR-127 silence up-regulated Mcl-1 expression. In conclusion, lncRNA ANRIL alleviated OGD-induced PC-12 cell injury as evidenced. PI3K/AKT pathway might be involved in this regulating progression.

Introduction
Ischaemic stroke (IS), which accounts for approximately 85% of all strokes is caused by immediate deprivation of both glucose and oxygen caused by the rapid loss in blood flow to brain [1,2]. Accumulating evidence revealed that IS becomes one of the leading cause of mortality and disability in adults all through the world [3]. Nowadays, the therapies for IS are intravenous alteplase administered within 4.5 h after symptom onset and mechanical thrombectomy [4,5]. However, shortcoming of plasminogen activator (alteplase) therapy urges researchers to seek new strategies or medicines. Better understanding of the underlying mechanisms about the pathology of stroke and post-stroke recovery might provide novel and effective approaches for the treatment of IS.

Long non-coding RNAs (lncRNAs) are reported to play important roles in regulation of gene transcription in diverse biological processes, such as apoptosis, invasion, histone modification, regulation of mRNA splicing and as a sink for some miRNAs [6–9]. Importantly, lncRNAs, which are also abundant in the central nervous system, have been implicated in brain development [10]. Although the expression and functions of lncRNAs in stroke and neuroprotection remain not to be clarified [11], increasing evidence have prove that lncRNAs are closely involved in IS, such as maternally expressed gene 3 (MEG3), H19, CaMK2D-associated transcript 1 (C2dat1), Fos downstream transcript (FosDT), small nucleolar RNA host gene 14 (SNHG14), and taurine-upregulated gene 1 (TUG1) which have been found to affect cell apoptosis, inflammation, cell death, and angiogenesis in IS [12]. For example, previous study reported that IncRNA antisense non-coding RNA in the INK4 locus (ANRIL) has been related with a higher risk of developing cardiovascular events and ANRIL was demonstrated to be associated with IS [13]. Meanwhile, ANRIL was reported to play a role in inflammatory responses and atherosclerosis [13,14]. Moreover, one former study demonstrated that IncRNA ANRIL altered the expressions of vascular endothelial growth factor (VEGF) and apromoted ngiogenesis in rats [15], which provided potential effects of ANRIL in IS. Based on these valuable findings reported by previous studies, we aimed to explore the potential roles of IncRNA ANRIL in IS cells.

In addition, former literature demonstrated that IncRNAs can directly bind to miRNA and regulate degradation of miRNA [12]. miRNAs are consist of short sequences of nucleotides (approximately 21–23) that regulate gene expression at the post-transcriptional level via directly effects on the 3′-untranslated region of mRNAs, leading to translational repression or mRNA degradation [16]. miRNAs are abundant...
in the nervous system, where they functioned as critical regulators of neuronal development [17]. Moreover, several miRNAs have been shown to be involved in stroke-related biological processes [18]. Among all these identified miRNAs, silence of miR-127 was reported to protect PC-12 cells from lipopolysaccharide(LPS)-induced inflammatory injury [19]. In addition, miR-127 was observed to be upregulated in atherosclerotic stroke [20]. Therefore, further experiment was performed to investigate whether miR-127 participated in IS with ANRIL.

In our study, we used oxygen-glucose deprivation (OGD) to stimulate PC-12 cells to establish cell model in vitro. We explored the effects of ANRIL on OGD-treated PC-12 cells. Our study might provide a better understanding about IS and provide a base foundation for seeking for the better therapies for IS.

**Materials and methods**

**Cell culture**

PC-12 cells (a rat adrenal medulla-derived pheochromocytoma cell line) were purchased from Kunming Institute of Zoology (Kunming, China). Cells were seeded into flasks (density of 1 × 10^5 cells/ml) in Dulbecco’s minimal Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS, Life Science, UT, USA), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were cultured at 37 °C in a humidified incubator with 5% CO₂. LY294002 (10 µM) was used as an inhibitor of the phosphatidylinositol 3'-kinase (PI3K)/protein kinase B (AKT) signalling pathway.

**Oxygen and glucose deprivation model**

For OGD treatment, culture medium was replaced by a glucose-free DMEM without FBS and cells were placed in an anaerobic, temperature controlled (37 ± 0.5 °C) chamber flushed with 5% CO₂ and 95% N₂ (v/v) for various time intervals (0, 2, 4, 6 and 8 h). After treatment for these time intervals, cells were replaced with normal medium containing 10% FBS and returned to the incubator under normoxic condition (37 °C, 5% CO₂). The control groups were treated similarly except for exposure to OGD [21]

**Cell counting kit-8 (CCK-8) assay**

Cells were seeded in 96-well plates (5000 cells/well) and cell viability was determined using CCK-8 (Dojindo Molecular Technologies, Gaithersburg, MD, USA). Briefly, after stimulation, CCK-8 solution was added to the culture medium with or without glucose, and the cells were incubated for 1 h at 37 °C in humidified incubator with 95% air or 95% N₂. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

**Apoptosis assay**

Apoptosis was determined by flow cytometric analysis of cells stained using an annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (Beijing Biosea Biotechnology, Beijing, China) according to the manufacturer’s instructions. Cells (100,000 cells/well) were seeded in 6-well plate. Treated cells were washed twice with cold PBS and resuspended in buffer. Cells (adherent and non-adherent) were combined and treated according to the manufacturer’s instruction and detected using flow cytometer (Beckman Coulter, USA) to differentiate apoptotic cells (annexin-V positive/PI-negative) from necrotic cells (annexin-V-negative/PI-positive).

**Transfection**

The full-length ANRIL and Mcl-1 sequences and short-hairpin RNA directed against Mcl-1 were cloned into pcDNA™3.1, pEX-2 and U6/GFP/Neo plasmids (GenePharma), respectively; these constructs were designated as pcDNA3.1-ANRIL, siANRIL, pEX-Mcl-1 and sh-Mcl-1, respectively. The ANRIL coding sequence was amplified by PCR and subcloned into pcDNA vector to construct recombinant plasmid pcDNA3.1-ANRIL. The concentration of pcDNA was 50 nM, and the sequence of si-ANRIL1 was 5’-GGAATAAGGAGGACACAGTGAG-3’ with siRNA final concentration of 50 nM. The sequence of sh-Mcl-1 5’-GAAACAAATGTGTTTATGAA-3’ with the final concentration was 50 nM. Plasmid carrying a non-targeting sequence was used as a negative control for sh-Mcl-1; this construct was designated as sh-negative control. The stably transfected cells were selected using culture medium containing 0.5 mg/ml G418 (Sigma-Aldrich, St Louis, MO, USA). After approximately 4 weeks, G418-resistant cell clones were established.

miR-127 mimic, miR-127 inhibitor and the negative controls were synthesized by GenePharma Co. (Shanghai, China). Cell transfections were conducted using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer’s protocol. The rno-miR-127 mimic concentration was 50 nM and miR-127 inhibitor concentration was 150 nM. The mimic sense was 5’-UGGAUCCGUCUGCUUGGCU-3’ and mimic antisense was 5’-CCAAGCUCAGGGAGGAUCCGAUU-3’ and inhibitor was 5’-ACAG CAAG CUCA GAGC GAUC CGA-3’. 

**Quantitative real-time polymerase chain reaction (qRT-PCR) analysis**

Total RNA was extracted from cells using TRIzol reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer’s instructions. The One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) was used for Real-Time PCR analysis to determine the expression level of ANRIL and Mcl-1. miRNA expression was measured using the TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal Master Mix II with the TaqMan mRNA Assay of miR-127 (Applied Biosystems, Foster City, CA, USA). GAPDH and U6 were used as the internal controls.
Western blot analysis

Total proteins for western blot analysis were extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA) according to the manufacturer’s instructions. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Bio-Rad Bis-Tris Gel system according to the manufacturer’s instructions and then transferred to a polyvinylidene difluoride (PVDF) membrane using the Bio-Rad ChemiDoc™ XRS system according to the manufacturer’s protocol. Membranes were incubated with primary antibodies at 4 ℃ overnight. Primary antibodies included: anti-pro-caspase-3 antibody (ab13847), anti-cleaved-caspase-3 antibody (ab49822), anti-Bcl-2-associated X protein (Bax) antibody (ab32503), anti-B-cell lymphoma 2 (Bcl-2) antibody (ab196495), anti- cytochrome C (cyto C) antibody (ab133504), anti-GAPDH antibody (ab181602), anti-myeloid cell leukemia 1 (Mcl-1) antibody (ab32087) all from Abcam (Cambridge, UK); anti-p-PI3K antibody (4228), anti-t-PI3K antibody (4249), anti-p-AKT antibody (4950), anti-t-AKT antibody (4691), from Cell Signaling Technology (Beverly, MA, USA). Primary antibodies were prepared in 5% blocking buffer and diluted according to products instructions. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. GAPDH served as a loading control. After rinsing, immunoreactive bands were detected using the Immobilon Western Chemiluminescent horseradish peroxidase (HRP) Substrate (Millipore, MA, USA). The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

Statistical analysis

Statistical analyses were performed using SPSS 19.0 statistical software SPSS (Chicago, IL, USA). Data were presented as the mean ± standard deviation (SD) of three independent experiments. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by post hoc Tukey’s tests. p < .05 was considered as statistical significance.

Results

OGD induced PC-12 cell injury and decreased ANRIL expression

To investigate the role of ANRIL in IS, OGD was used to stimulate PC-12 cells to establish a cell model of IS in vitro. PC-12 cells were subjected to OGD in different treatment time intervals (0, 2, 4, 6 and 8 h) and the effects of OGD on cell viability were detected. OGD induced reduction of cell viability in a time-dependent manner (p < .05, Figure 1(A)). In contrast, OGD treatment led to increasing apoptosis in a time-dependent manner (p < .05, Figure 1(B)). Furthermore, western blot analysis demonstrated that expression of the anti-apoptotic protein Bcl-2 was down-regulated, while expression levels of pro-apoptotic protein, Bax, Cyto C and cleaved-Caspase-3 were increased with the increasing of time intervals (p < .05, Figure 1(C–D)). ANRIL expression was reported to be closely correlated with IS [14]; therefore, we determined the expression of ANRIL in OGD-treated cells. qRT-PCR analysis showed that ANRIL expression was significantly down-regulated when PC-12 cells treated with OGD in a time-dependent manner (p < .05), indicating that ANRIL might play important roles in PC-12 cells response to OGD treatment (Figure 1(E)).

Dysregulation of ANRIL affected OGD-induced PC-12 cell injury

To investigate the role of ANRIL in OGD-induced injury, PC-12 cells were transfected with pc-ANRIL, si-ANRIL and their corresponding negative controls. Cells co-transfected with pc-ANRIL and si-ANRIL revealed significantly higher and lower levels of ANRIL expression compared with control under OGD treatment, indicating high transfection efficiency (p < .05, Figure 2(A) and (F)). Further analysis showed that OGD-treated PC-12 cells transfected with the pcDNA3.1 resulted in a significant decrease in cell viability (p < .05), while that this effect was significantly ameliorated by transfection with pcDNA3.1-ANRIL (p < .05; Figure 2(B)). In addition, transfection with pDNA3.1-ANRIL significantly decreased cell apoptosis compared with transfection with pcDNA3.1 under OGD treatment (p < .05; Figure 2(C)). Western blot analysis in Figure 2(D–E) with up-regulating Bcl-2, and down-regulating Bax, Cyto and cleave-Caspase-3 by ANRIL overexpression compared with transfection with pcDNA3.1 confirmed the result in Figure 2(C). Interestingly, further researches about transfection with si-ANRIL led to the opposite results in cell viability and cell apoptosis compared with transfection with pcDNA3.1-ANRIL (p < .05, Figure 2(G–J)). Taken together, overexpression of ANRIL promoted OGD-treated PC-12 cell growth, while silence of ANRIL decreased OGD-treated PC-12 cells growth.

Overexpression of miR-127 promoted OGD-induced PC-12 cell injury while suppression of miR-127 alleviated the cell injury

miR-127 was reported to be overexpressed in atherosclerotic [20] and was a potential biomarker or therapeutic target for stroke [22]. Therefore, we inferred that miR-127 might be involved in the effects of ANRIL on OGD-treated PC-12 cells. As shown in Figure 3, transfection with miR-127 mimic and inhibitor without OGD treatment could significantly alter the expression of miR-127 in PC-12 cells (both p < .01, Figure 3(A)). miR-127 mimic or inhibitor were then transfected to investigate the role of miR-127 in OGD-treated PC-12 cells. Compared with PC-12 cells transfected with scramble, miR-127 overexpression was observed following transfection with the miR-127 mimic and down-expression of miR-127 by transfection with miR-127 inhibitor, which indicated high transfection efficiency (p < .05, Figure 3(B)).
We next investigated the effects of transfection with miR-127 mimic and inhibitor on OGD-induced cell injury. Compared with the effects of controls, transfection with the miR-127 mimic significantly decreased cell viability \((p < .05)\) and increased cell apoptosis \((p < .05)\) compared with transfection with scramble in OGD-stimulated PC-12 cells (both \(p < .05\); Figure 3(C and D)). In contrast, transfection with miR-127 inhibitor significantly promoted OGD-induced cell growth through increasing cell viability and decreasing cell apoptosis \((p < .05\); Figure 3(C and D)). Western blot analysis showed that transfection with miR-127 mimic inhibited Bcl-2 expression while up-regulated Bax and Cyto C and cleaved-caspase-3 expression. However, transfection with miR-127 inhibitor led to the opposite results in Figure 3(E–F). These results demonstrated that miR-127 overexpression promoted OGD-induced cell injury while miR-127 down-regulation reduced OGD-induced cell injury.

LncRNA ANRIL reduced OGD-induced cell injury through down-regulation of miR-127

To clarify the relationship between LncRNA ANRIL and miR-127, further experiments were performed. Firstly, we investigated the expression of miR-127 by different ANRIL expression. Results showed that miR-127 expression was...
Figure 2. Overexpression of ANRIL reduces oxygen and glucose deprivation (OGD)-induced PC-12 cell injury while silence of ANRIL led to the opposite results. PC-12 cells were transfected with the pc-ANRIL and si-ANRIL and a stably expressing cell line was selected; un-transfected cells and cells transfected with the empty vector (pcDNA3.1) served as negative controls. (A and F) qRT-PCR analysis of ANRIL expression. The effects of ANRIL overexpression on cell injury induced by exposure to OGD (6 h) were then evaluated. (B and G) A Cell Counting Kit-8 (CCK-8) assay of cell viability; (C and H) Flow cytometric analysis of apoptosis by annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining; (D–E and I–J) Western blot analysis of apoptosis-related protein expression; GAPDH served as the loading control. Data represent the mean ± standard deviation (SD) (n = 3) of three independent experiments. *p < .05.
down-regulated by transfection with pcDNA3.1-ANRIL as relative to transfection with pcDNA3.1 in OGD-treated cells ($p < .05$) while silence of ANRIL led to the opposite results ($p < .05$, Figure 4(A)), which suggested a negative relationship between miR-127 and ANRIL. Then, we found that co-transfection with pc-ANRIL and miR-127 mimic up-regulated the expression of miR-127 (Figure 4(B)). Interestingly, co-transfected with pc-ANRIL impaired the protective functions of ANRIL in OGD-treated PC-12 cells by decreasing cell viability ($p < .05$, Figure 4(C)) and increasing cell apoptosis ($p < .05$, Figure 4(D–F)). This result revealed that ANRIL alleviated OGD-induced cell injury by down-regulation of miR-127.

miR-127 negatively regulated the expression of mcl-1

Mcl-1 is a potent anti-apoptotic member of the Bcl-2 family of proteins located in the mitochondria [23]. Overwhelming
reports about various RNAs targeting Mcl-1 to achieve their functions, such as miR-137 and miR-197 [24], miR-17-5p [25], miR-320 [26]. Importantly, Mcl-1 was reported to be involved in cerebral ischaemia [27]. Therefore, we hypothesized that Mcl-1 was also modulated by miR-127 in PC-12 cells. To evaluate this hypothesis, Mcl-1 expression by transfection with the miR-127 mimic or inhibitor was determined at the mRNA and protein levels (Figure 5). Compared with the group transfected with scramble or negative control, Mcl-1 expression was statistically decreased by transfection with the miR-127 mimic, while Mcl-1 expression was significantly increased by transfection with miR-127 inhibitor at in both the mRNA and protein levels (both \( p < .05 \), Figure 5(A–B)).

On the other hand, we investigated the role of ANRIL in the mechanism of miR-127 negatively regulates Mcl-1 expression. As shown in Figure 5(C–D), transfection with pcDNA3.1-ANRIL increased Mcl-1 expression at both the mRNA (\( p < .05 \)) and protein levels (\( p < .05 \)) in OGD-treated cells, while this effect was abrogated by co-transfection with pcDNA3.1-ANRIL and the miR-127 mimic (\( p < .05 \)). These observations indicated that ANRIL up-regulated the expression of Mcl-1 by down-regulation of miR-127.

Figure 4. ANRIL protected against oxygen and glucose deprivation (OGD)-induced cell injury through down-regulation of miR-127. (A–B) the expression of miR-127 was detected by qRT-PCR. Then effects of co-transfection with pc-ANRIL and miR-127 mimic was detected by (C) Cell Counting Kit-8 (CCK-8) assay of cell viability; (D) Flow cytometric analysis of apoptosis by annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining; (E–F) Western blot analysis of apoptosis-related protein expression; GAPDH served as the loading control. Data represent the mean ± standard deviation (SD) (\( n = 3 \)) of three independent experiments. *\( p < .05 \).
miR-127 silence up-regulated mcl-1 expression through activation of PI3K/AKT signal pathways

Previous study pointed out that PI3K/AKT pathway was closely involved in ischaemic stroke [28]. Besides, miR-127-5p was reported to achieve its functions through regulating PI3K/AKT pathway [29]. Therefore, we intended to explore whether effects of miR-127 on OGD-treated PC-12 cells was also through PI3K/AKT pathway. OGD was found to reduce the levels of the phosphorylated (active) forms of PI3K and AKT compared with the levels detected in the absence of OGD (Figure 6(A–B)). Compared with OGD treatment alone, transfection with miR-127 mimic markedly enhanced the OGD-induced reduction in the levels of p-PI3K and p-AKT (Figure 6(A–B)). In contrast, the miR-127 inhibitor was associated with an extremely marked increase in the levels of p-PI3K and p-AKT. As shown in Figure 6(A and B), these effects were inhibited by the PI3K inhibitor, LY294002, which also abolished the ability of the miR-127 inhibitor protect against the OGD-induced reduction in Mcl-1 expression.

Overexpression of mcl-1 reduced OGD-induced PC-12 cell injury, while suppression of mcl-1 promoted the cell injury

To clarify the role of Mcl-1 in OGD-induced injury, we generated cell lines exhibiting Mcl-1 overexpression or suppression by transfection with the pEX-Mcl-1 or sh-Mcl-1 in OGD-stimulated cells, respectively. The up-regulation of Mcl-1 by transfection with pEX-Mcl-1 while down-regulation of Mcl-1 by transfection with sh-Mcl-1 in both RNA and protein level indicated high transfection efficiency in OGD-treated cells ($p < .05$, Figure 7(A–B)). In addition, results showed that Mcl-1 overexpression significantly promoted OGD-induced cell growth by increasing cell viability and decreasing cell...
apoptosis, while Mcl-1 suppression exacerbated OGD-induced cell injury \( (p < .05, \text{Figure 7(C and D)}) \). Furthermore, western blot analysis showed that Mcl-1 overexpression markedly induced Bcl-2 expression and decreased expression of Bax, Cyto C and cleaved-caspase-3, while Mcl-1 suppression led to the opposite result in OGD-treated cells (Figure 7(E–F)). These results demonstrated that Mcl-1 overexpression reduced OGD-induced cell injury.

**Discussion**

In the present study, we investigated the effects of ANRIL on OGD-induced injury in PC-12 cells to further elucidate the underlying mechanism about the potential pathology of IS (Figure 8). We found that ANRIL overexpression alleviated OGD-induced cell injury by increasing cell viability and decreasing cell apoptosis in PC-12 cells. In addition, ANRIL negatively regulated the expression of miR-127, which negatively modulated by Mcl-1 expression. Meanwhile, miR-127 was involved in regulating PI3K signal pathway. Our study firstly constructed a regulating relationship between lncRNA and miR-127 in PC-12 cells, which provided a novelty insight in understanding the regulating system in ischemic stroke.

Increasing evidence indicated important roles of lncRNAs, which are abundantly expressed in the nervous system, in responses to a variety of stress conditions such as heat shock, DNA damage, hypoxia, nutrient limitation, lipid-induced oxidation, and infection [30,31]. Notably, among these identified lncRNAs, ANRIL received considerable attention due to its important functions in regulating biological activities. For example, study from Zhang et al. demonstrated that up-regulation of lncRNA ANRIL increased VEGF expression level and enhanced angiogenesis of diabetes mellitus along with cerebral infarction by activating NF-xB signalling pathway in a rat model [15]. Importantly, ANRIL expression was reported to be associate with stroke [32], especially in IS [14,33]. Thus, in this study, we investigated the potential role of ANRIL in IS using an OGD-induced cell model.

Previous study reported that ANRIL may serve as a novel genetic marker for the risk of atherothrombotic and haemorrhagic stroke [32]. In addition, ANRIL knock-down will inhibit cell proliferation *in vitro* [34]. Interestingly, similar results were also observed in our study that transfection with si-ANRIL reduced cell growth by decreasing cell viability and increasing cell apoptosis.

Accumulating evidence indicates the existence of cross-regulation between miRNAs and lncRNAs in the control of mammalian gene expression [35]. Since miR-127 has been reported to increase apoptosis of primary cultured spinal neurons [36], we investigated a potential role of miR-127 cooperated with ANRIL to protect against OGD-induced injury. Surprisingly, results showed that ANRIL negatively regulated the expression of miR-127 in PC-12 cells, indicating that miR-127 might be involved in. Moreover, overexpression of miR-127 promoted OGD-induced PC-12 cell injury while suppression of miR-127 alleviated the cell injury. In addition, miR-127 overexpression impaired the protective function of ANRIL overexpression indicated that effects of ANRIL on OGD-induced cell injury were through regulation of miR-127. This result was consistent with miR-127 promoted lung inflammation and injury [37] and knockdown of ANRIL aggravated H2O2-induced injury in PC-12 cells [38]. However, contrary results were also reported that miR-127 overexpression protected actin cytoskeleton from disorganization provoked by hypoxic injury [39]. Taken together, miR-127 might have different functions in different diseases.

Our results suggested that ANRIL overexpression protects against OGD-induced cell injury shown as decreasing cell apoptosis and anti-apoptotic protein. Mcl-1 was reported to be target of various miRNAs. Hence, further researches were performed to investigate whether Mcl-1 was involved in the protective function of ANRIL through regulation of miR-127. Moreover, Mcl-1 is implicated as a critical pro-survival protein.
in a vast array of different cell types [40] and ubiquitin-mediated proteolysis is required for initiation of the mitochondrial apoptotic cascade [41]. Our results showed that miR-127 negatively regulated Mcl-1 expression; thus, it can be speculated that the effects of ANRIL protected against OGD-induced injury by down-regulation of miR-127, which abrogates the negative regulatory effects on Mcl-1 expression. Therefore, we inferred that up-regulation of Mcl-1 inhibited apoptosis leading to protection against the apoptogenic signals associated with ischemia. This inference was further confirmed by the observation that the protective effects of Mcl-1 were enhanced by Mcl-1 overexpression and inhibited by Mcl-1 silencing.

PI3K/AKT pathway was found to exert an important role in stroke [28,42]. Importantly, PI3K/AKT was also reported to be closely correlated with IS [43]. In addition, previous study revealed that miR-127-5p mimic significantly inhibited the activation of PI3K/AKT pathway [44]. Hence, in our study, we investigated the role of miR-127 and PI3K/AKT pathway. Results demonstrated that miR-127 suppression leading to
up-regulated Mcl-1 expression which was mediated via the PI3K/AKT pathway. Our study confirmed the potential role of PI3K/AKT in OGD-induced cell injury.

Conclusion

In this study, we demonstrated that the lncRNA ANRIL alleviated OGD-induced cell injury as evidenced by increasing cell viability and decreasing cell apoptosis through suppressing miR-127 expression which negatively regulated by Mcl-1 expression. There were network or cascade reactions among ANRIL, miR-127 and Mcl-1 to regulate OGD-induced PC-12 cell injury. Our study based on the in vitro cell model to study the effects of IncRNA ANRIL, more in vivo studies are needed to confirm the results and further provide data for the clinical IS treatment.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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