Research Paper

Oleuropein Inhibits Astrocyte Damage Induced by Oxygen-Glucose Deprivation

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Zhang et al.: Mechanism of Oleuropein on the Oxidative Damage and Apoptosis of Astrocytes

To investigate the effect and its mechanism of oleuropein on the oxidative damage and apoptosis of astrocytes induced by oxygen-glucose deprivation, and its effect on circ_TTC3 and microRNA-138-5p. Astrocytes were divided into control group, oxygen-glucose deprivation group, oxygen-glucose deprivation+oleuropein 25 μmol/l, 50 μmol/l, 100 μmol/l groups, oxygen-glucose deprivation+si-negative control, si-circ_TTC3 group, oxygen-glucose deprivation+oleuropein+plasmid cloning deoxyribonucleic acid plasmid cloning deoxyribonucleic acid and plasmid cloning deoxyribonucleic acid-circ_TTC3 group. In comparison with the control group, the inhibiting rate of cell growth, apoptosis rate, malondialdehyde, lactate dehydrogenase levels and circ_TTC3 expression of astrocytes in the oxygen-glucose deprivation group were increased, while superoxide dismutase activity and miR-138-5p expression were declined (p<0.05). Compared to the oxygen-glucose deprivation group, the proliferative inhibiting rate, apoptosis rate, malondialdehyde, lactate dehydrogenase levels and circ_TTC3 expression of astrocytes in the oxygen-glucose deprivation+oleuropein 25 μmol/l group, oxygen-glucose deprivation+oleuropein 50 μmol/l group, and oxygen-glucose deprivation+oleuropein 100 μmol/l group were all weakened, superoxide dismutase activity and microRNA-138-5p expression were enhanced (p<0.05), and all were concentration-dependent. After silencing circ_TTC3, circ_TTC3 expression, malondialdehyde, lactate dehydrogenase levels, cell proliferation inhibition rate and apoptosis rate in astrocytes in the oxygen-glucose deprivation+si-circ_TTC3 group were all lower than those in oxygen-glucose deprivation+si-negative control group, while the superoxide dismutase activity was higher than that in oxygen-glucose deprivation+si-negative control group (p<0.05). Circ_TTC3 targeted and regulated microRNA-138-5p. Circ_TTC3 level, malondialdehyde and lactate dehydrogenase levels, cell proliferation inhibition rate, and apoptosis rate in astrocytes in the oxygen-glucose deprivation+oleuropein+ plasmid cloning deoxyribonucleic acid-circ_TTC3 group were all higher than those in oxygen-glucose deprivation+oleuropein+plasmid cloning deoxyribonucleic acid group, and there was a decrease of superoxide dismutase activity in the oxygen-glucose deprivation+oleuropein+plasmid cloning deoxyribonucleic acid-circ_TTC3 group compared with oxygen-glucose deprivation+oleuropein+plasmid cloning deoxyribonucleic acid group (p<0.05). Oleuropein could reduce oxidative stress and apoptosis, thereby protecting astrocytes from damage induced by oxygen-glucose deprivation by upregulating circ_TTC3 to target microRNA-138-5p.

Key words: Astrocytes, oleuropein, oxygen-glucose deprivation, oxidative stress, apoptosis, circ_TTC3, microRNA-138-5p

Stroke is a main reason of physical disabilities and the second-major reason for death worldwide[1,2], therein approximately 87 % of these cases are attributed to ischemia[3]. During the past decades, researchers have primarily concentrated upon neurons to treat ischemic injuries. Ischemic injury usually involves multiple pathological processes, including oxidative stress and apoptosis[4,5]. There is increasing evidence about the main function of astrocytes in ischemic damage. Astrocytes can supply the support for neurons at nutritional, structural and metabolic levels and they are the richest sort of neuroglial cells in the brain[6]. Aside from these physical functions, these astrocytes

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also can play neuroprotective role in fighting ischemic damage\cite{7,8}. Injury and unbalance of the astrocytes will compromise the survival of neurons after ischemia. Hence, protecting and functionally regulating astrocytes is crucial tactic for preventing neuronal injury ascribed to ischemia.

Oleuropein is a phenolic compound from olive leaf extract. According to literature reports, oleuropein has been reported to have good therapeutic effects on the damage caused by myocardial ischemia-reperfusion\cite{9}, cerebral ischemia-reperfusion\cite{10} and renal ischemia-reperfusion\cite{11} through antioxidant or anti-apoptotic mechanisms. However, the role of oleuropein in ischemia-damaged astrocytes is not yet clear.

Some circular RNAs (circRNAs) and microRNAs (miRNAs) have been identified as key regulatory Ribonuclic Acid (RNAs) participated in the occurrence and evolution of ischemic stroke\cite{12,13}. Circ_TTC3 was mentioned to promote cerebral ischemia-reperfusion damage and inhibit neural stem cell function in cerebral infarction through miR-372-3p/Toll-Like Receptor 4 (TLR4) axis\cite{14}. miR-138-5p was low expressed in PC-12 cells which was treated with Oxygen-Glucose Deprivation (OGD)/reoxygenation\cite{15}, and can also protect astrocytes from damage induced by OGD\cite{16}. However, the relation of circ_TTC3 and miR-138-5p and whether it is involved in the functional mechanism of oleuropein-induced ischemic injury of astrocytes are still unclear. In view of this, this research concentrate upon investigating the effects of oleuropein on apoptosis and oxidative stress damage of astrocytes induced by OGD, and combined with its regulatory effects on circ_TTC3 and miR-138-5p, furtherly to determine the latent molecular mechanism underlying the efficacy of oleuropein.

**MATERIALS AND METHODS**

**Cells and reagents:**

Human astrocytes were purchased from two Biotech Co., Ltd (Shenzhen, China). Oleuropein (≥98 %), Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Lactate Dehydrogenase (LDH) kits were bought from Beyotime (Shanghai, China).

**Cell culture and OGD injury:**

The astrocytes were fostered at 37° in Dulbecco’s Modified Eagle Medium (DMEM) added with 10 % Fetal Bovine Serum (FBS) and 1 % penicillin/streptomycin antibiotics in 5 % Carbon dioxide (CO\textsubscript{2}) incubator. For OGD injury, the astrocytes were quickly cleaned with Phosphate Buffer Solution (PBS) followed by the addition of sugar-free medium, and later injured in 37° atmosphere with 5 % CO\textsubscript{2}, 1 % Oxygen (O\textsubscript{2}) and 94 % Nitrogen (N\textsubscript{2}) for 6 h\cite{17}.

**Experimental grouping and treatment:**

The experiment was split into multiple groups; control, OGD, OGD+oleuropein 25 μmol/l, OGD+oleuropein 50 μmol/l, OGD+oleuropein 100 μmol/l, OGD+si-Negative Control (si-NC), OGD+si-circ_TTC3, OGD+oleuropein+plasmid cloning Deoxyribonucleic Acid (pcDNA), OGD+oleuropein+pcDNA-circ_TTC3 group. MiR-NC or miR-138-5p mimic, si-circ_TTC3, si-NC, pcDNA-circ_TTC3, pcDNA were purchased from Ribobio (Guangzhou, China). Among them, the astrocytes in the control group did not undergo any treatment, and the cells in the OGD group were damaged by oxygen and glucose deprivation.

Oleuropein was dissolved in PBS and prepared as 10 mmol/l mother solution. During the experiment, astrocytes were treated with (25, 50, or 100 μmol/l) oleuropein diluted by culture medium for 24 h and oxygen-sugar deprivation injury was performed. Astrocytes in the groups of OGD+si-NC, OGD+si-circ_TTC3, OGD+oleuropein+pcDNA, and OGD+oleuropein+pcDNA-circ_TTC3 were planted into 6-well plates (1×10\textsuperscript{5} cells). According to the instruction manual of Lipofectamine 2000, si-NC, si-circ_TTC3, pcDNA and pcDNA-circ_TTC3 were transfected into cells with 60 % confluence. After (6-8) h, above cells were replaced with complete medium or 100 μmol/l oleuropein-added medium for 24 h, followed by OGD injury.

**Cell Counting Kit-8 (CCK-8) assay:**

Astrocytes were planted in 96-well plate and managed as mentioned in experimental grouping and treatment. Later, CCK-8 reagent (10 μl/well, Beyotime) was supplied for the cells and put into a 5 % CO\textsubscript{2} incubator for 2 h. Synergy H1 microplate reader was used for measuring the (Optical Density (OD)=450 nm) value. The proliferation inhibition rate of astrocytes was 100 %-(OD value ratio of treatment group/control group)×100 %.
Analysis of apoptosis:
The astrocytes were grouped and processed as described in experimental grouping and treatment. Following the wash using pre-cooling PBS, $1 \times 10^5$ cells were gathered and dyed for 15 min using Annexin V-Fluorescein Isothiocyante (FITC) and Propidium Iodide (PI) in a gloomy environment following apoptotic kit (Beyotime). The apoptotic percentage was measured via flow cytometry and cell quest software.

Detection of oxidative stress levels:
The astrocytes were grouped according to experimental grouping and treatment and the supernatant of the cells was respectively collected according to the operating instructions of the SOD, MDA, and LDH detection kits, and later the SOD activity, MDA and LDH levels of astrocytes were tested by corresponding kits.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR):
The astrocytes were grouped and processed as described in experimental grouping and treatment. Trizol (Invitrogen, Carlsbad, CA, United States of America (USA)) was used for isolating total RNA, and multi scribe RT kit (Invitrogen) was applied for reverse transcription. The levels of circ_TTC3 and miR-138-5p was respectively tested via SYBR Green Master Mix (TaKaRa, Dalian, China) and miRNA qPCR kit (Ribobio). Relative expression was evaluated through $2^{-\Delta\Delta C_{t}}$ strategy. The primers are listed as, forward circ_TTC3, 5'-CACGATTGCATCCCTGTGTG-3'; reverse 5'-ACTGTCACGTTTCAAGATCACT-3'; forward Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), 5'-AAGCCACCCCACTTCTCTCAA-3'; reverse 5'-AATGCTATCACCTCCCCTGTG-3'; forward miR-138-5p, 5'-GCCGGATAAGTGTTGTGGTCGA-3'; reverse 5'-ACTGAGCAAGCACTACCACCAGCA-3'; forward U6, 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'.

Dual-luciferase reporter assay:
Starbase software forecasted the binding sequences of circ_TTC3 and miR-138-5p. The Wild-Type (WT) and Mutant (MUT) circ_TTC3 sequences with miR-138-5p combinative sites were synthesized and fused with the luciferase reporter plasmid psiCHECK2 to construct WT-circ_TTC3 and MUT-circ_TTC3 plasmids. The astrocytes were inoculated into 6-well plates, and WT-circ_TTC3 or MUT-circ_TTC3 was subjected to the co-transfection of miR-NC or miR-138-5p mimics according to the manual of Lipofectamine 2000. 48 h later, the activity of luciferase was tested through an exploratory system of dual-luciferase reporter gene. In addition, si-NC, si-circ_TTC3, pcDNA or pcDNA-circ_TTC3 was respectively transfected into astrocytes, and 48 h later, miR-138-5p level in astrocytes was tested as described in qRT-PCR.

Statistical analysis:
Data are appeared as mean (x ± s) standard deviation. Statistical Package for the Social Sciences (SPSS) 22.0 software was used to evaluate statistical significance. Comparing the difference of multiple groups or between two groups was conducted using one-way analysis of variance or independent sample t-test. p<0.05 was be deemed to have statistical significance.

RESULTS AND DISCUSSION
Compared with control group, the rate of proliferation inhibition and apoptosis of astrocytes in OGD group was upregulated (p<0.05). While the rate in OGD+oleuropein (containing 25, 50, or 100 μmol/l oleuropein) was declined vs. OGD group (p<0.05) in a concentration-related way (Table 1 and fig. 1).

SOD activity of astrocytes after OGD treatment was reduced vs. control group, MDA and LDH levels were incremental (p<0.05). In comparison with OGD group, SOD activity of astrocytes after the treatment of OGD and different concentration of oleuropein (25, 50, or 100 μmol/l) was increased, while MDA and LDH levels were decreased (p<0.05), and all in a concentration-related way (Table 2).

Compared with control group, circ_TTC3 level in astrocytes of OGD group was increased, while miR-138-5p level was declined (p<0.05). While the expression of circ_TTC3 in astrocytes of OGD and 25, 50, or 100 μmol/l oleuropein co-processing group was decreased vs. OGD group, and the expression of miR-138-5p was increased (p<0.05), and both of them were concentration-
TABLE 1: THE INFLUENCE OF OLEUROPEIN ON APOPTOSIS OF ASTROCYTES (x̄±s, n=9)

<table>
<thead>
<tr>
<th>Group</th>
<th>Inhibition rate/%</th>
<th>Apoptosis rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00±0.00</td>
<td>7.32±0.79</td>
</tr>
<tr>
<td>OGD</td>
<td>56.26±1.80*</td>
<td>24.44±1.65*</td>
</tr>
<tr>
<td>OGD+oleuropein 25 μmol/l</td>
<td>44.78±2.16*</td>
<td>21.39±1.22*</td>
</tr>
<tr>
<td>OGD+oleuropein 50 μmol/l</td>
<td>31.93±1.15*</td>
<td>17.08±1.20*</td>
</tr>
<tr>
<td>OGD+oleuropein 100 μmol/l</td>
<td>21.11±1.45*</td>
<td>11.72±1.05*</td>
</tr>
<tr>
<td>F</td>
<td>1873.976</td>
<td>296.369</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: *p<0.05 vs. control group; #p<0.05 vs. OGD group; Δp<0.05 vs. OGD+oleuropein 25 μmol/l group; □p<0.05 vs. OGD+oleuropein 50 μmol/l group

TABLE 2: EFFECTS OF OLEUROPEIN ON SOD, MDA AND LDH IN ASTROCYTES (x̄±s, n=9)

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD/U·l⁻¹</th>
<th>MDA/nmol·ml⁻¹</th>
<th>LDH/U·l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>508.47±32.95</td>
<td>38.44±3.59</td>
<td>155.89±15.37</td>
</tr>
<tr>
<td>OGD</td>
<td>140.73±13.03*</td>
<td>410.90±21.66*</td>
<td>751.37±37.38*</td>
</tr>
<tr>
<td>OGD+oleuropein 25 μmol/l</td>
<td>246.88±13.56*</td>
<td>350.64±11.55*</td>
<td>633.77±28.32*</td>
</tr>
<tr>
<td>OGD+oleuropein 50 μmol/l</td>
<td>371.21±13.44*</td>
<td>264.34±13.44*</td>
<td>471.52±35.76*</td>
</tr>
<tr>
<td>OGD+oleuropein 100 μmol/l</td>
<td>434.65±18.51*</td>
<td>150.35±12.57*</td>
<td>281.82±20.34*</td>
</tr>
<tr>
<td>F</td>
<td>431.666</td>
<td>1069.020</td>
<td>652.529</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: *p<0.05 vs. control group; #p<0.05 vs. OGD group; Δp<0.05 vs. OGD+oleuropein 25 μmol/l group and □p<0.05 vs. OGD+oleuropein 50 μmol/l group

TABLE 3: EFFECTS OF OLEUROPEIN ON EXPRESSION OF circ_TTC3 AND miR-138-5P IN ASTROCYTES (x̄±s, n=9)

<table>
<thead>
<tr>
<th>Group</th>
<th>circ_TTC3</th>
<th>miR-138-5p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>OGD</td>
<td>3.75±0.11*</td>
<td>0.13±0.02*</td>
</tr>
<tr>
<td>OGD+oleuropein 25 μmol/l</td>
<td>3.19±0.08*</td>
<td>0.33±0.04*</td>
</tr>
<tr>
<td>OGD+oleuropein 50 μmol/l</td>
<td>2.46±0.10*</td>
<td>0.55±0.05*</td>
</tr>
<tr>
<td>OGD+oleuropein 100 μmol/l</td>
<td>1.52±0.08*</td>
<td>0.78±0.07*</td>
</tr>
<tr>
<td>F</td>
<td>1670.841</td>
<td>574.263</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: *p<0.05 vs. control group; #p<0.05 vs. OGD group; Δp<0.05 vs. OGD+oleuropein 25 μmol/l group and □p<0.05 vs. OGD+oleuropein 50 μmol/l group
dependent (Table 3).

After circ_TTC3 was silenced, circ_TTC3 level in OGD-induced astrocytes was approximately 0.68 times lower than that in si-NC transfection group, and the activity of SOD was elevated vs. OGD+si-NC group, while MDA and LDH levels, inhibition rate of cell growth and the rate of apoptosis were all decreased vs. OGD+si-NC group (p<0.05) (Table 4 and fig. 2).

The sites between circ_TTC3 and miR-138-5p binding was predicted by star base (fig. 3). The luciferase activity of WT-circ_TTC3 co-transfected with miR-138-5p was dramatically declined vs. miR-NC transfection (p<0.05), while there was no marked difference in the luciferase activity of MUT-circ_TTC3 in miR-138-5p and miR-NC groups (p=0.604) (Table 5). The level of miR-138-5p was 4.25±0.13 in si-circ_TTC3 group in astrocytes, which was higher than 1.00±0.00 in si-NC group (t=75.000, p<0.05), and 0.21±0.02 in pcDNA-circ_TTC3 group, which was lower than 1.00±0.00 in pcDNA group (t=118.500, p<0.05).

Circ_TTC3 level in astrocytes of OGD+oleuropein+pcDNA-circ_TTC3 group was about 1.89 times higher compared to OGD+oleuropein+pcDNA group and SOD activity was lower vs. OGD+oleuropein+pcDNA group. Moreover, MDA and LDH levels, inhibition rate of cell growth and apoptosis rate in astrocytes of OGD+oleuropein+pcDNA-circ_TTC3 group were all elevated compared to OGD+oleuropein+pcDNA group (p<0.05) (Table 6 and fig. 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>circ_TTC3</th>
<th>SOD/U.l</th>
<th>MDA/nmol.ml</th>
<th>LDH/U.l</th>
<th>Inhibition rate/%</th>
<th>Apoptosis rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGD+si-NC</td>
<td>1.00±0.00</td>
<td>139.21±16.42</td>
<td>411.71±26.47</td>
<td>737.02±43.50</td>
<td>56.40±2.08</td>
<td>24.23±1.75</td>
</tr>
<tr>
<td>OGD+si-circ_TTC3</td>
<td>0.32±0.04*</td>
<td>460.79±28.97*</td>
<td>99.16±6.43*</td>
<td>230.35±18.62*</td>
<td>14.21±0.81*</td>
<td>9.12±0.58*</td>
</tr>
<tr>
<td>t</td>
<td>51.000</td>
<td>28.971</td>
<td>34.422</td>
<td>32.122</td>
<td>56.703</td>
<td>24.588</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: *p<0.05 vs. OGD+si-NC group

Fig. 2: Silencing circ_TTC3 inhibited OGD-induced astrocyte apoptosis

Fig. 3: Combined sites of circ_TTC3 and miR-138-5p
LDH and MDA can also be used as indicators for ischemic injury. Here, we showed that OGD caused the damage of astrocytes. Compared with control group, the astrocytes treated by OGD showed higher cell proliferation inhibition rate, higher apoptosis rate, higher MDA, LDH levels and lower SOD activity. This result was similar to previous studies\[25,26\], which indicated that the OGD model used in this study successfully simulated ischemic injury.

Oleuropein, a phenolic component of olive, has multifarious biologic activities, including anti-oxidation, anti-dyslipidemia, weight loss, anti-diabetes, anti-atherosclerosis, anti-hypertension, anti-inflammatory and liver protection\[27,28\]. Also, oleuropein was reported to have suppressive effects on neurodegenerative and neuropsychiatric disorders, through playing neuroprotective role

Astrocytes are the richest glial cells existed in the central nervous system\[18,19\]. Astrocytes are demonstrated to exert a crucial function in protecting neurons after stroke in many ways, including antioxidant activity, ion homeostasis, energy transfer, and neurotransmitter transport\[20,21\]. Thus, alleviating astrocyte damage is a promising direction for preventing excessive neuronal injury after stroke. In vitro OGD models of astrocytes have been widely used to simulate ischemic damage occurring in stroke\[22\]. The main factors causing ischemic stroke include oxidative damage, apoptosis, autophagy, inflammation, energy metabolism disorders, and so on\[23\]. Cellular defense in the brain involves endogenous protective enzymes, such as SOD, which are produced by astrocytes to protect neurons from oxidative stress\[24\]. In addition, oxidative stress markers LDH and MDA can also be used as indicators for ischemic injury. Here, we showed that OGD caused the damage of astrocytes. Compared with control group, the astrocytes treated by OGD showed higher cell proliferation inhibition rate, higher apoptosis rate, higher MDA, LDH levels and lower SOD activity. This result was similar to previous studies\[25,26\], which indicated that the OGD model used in this study successfully simulated ischemic injury.

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in induction of apoptosis and autophagy\cite{29}. According to the reports, oleuropein can ameliorate the enhancement of LDH and MDA and the reduction of SOD activity in the cerebral tissues of infarcted rats in a stroke rat model, providing neuroprotective effects on ischemic stroke through its antioxidant and antithrombotic activities\cite{30}. Oleuropein improves cognitive function after stroke by promoting histone acetylation and phosphorylation of cyclic Adenosine 3’,5’-Monophosphate (cAMP) responsive element binding protein in middle cerebral artery occlusion rats\cite{31}. In a rat intracerebral hemorrhage model established by collagenase injection, oleuropein treatment has shown to have overall relief of neurologic deficits and cerebral edema associated with intracerebral hemorrhage in a dose-dependent manner\cite{32}. In addition, oleuropein inhibited cell apoptosis through the Tyrosine Kinase B (Akt)/Glycogen Synthase Kinase 3 Beta (GSK-3β) pathway and upregulated neurotrophic factors after cerebral ischemia/reperfusion damage in rats, thereby producing a tutelar influence on cerebral ischemia\cite{33}. In this study, oleuropein with various doses (25, 50 and 100 μmol/l) could reduce the astrocyte damage induced by OGD in a dose-dependent way through promoting proliferation, inhibiting apoptosis and oxidative stress damage, which was in line with the former study. These results indicated that oleuropein could reverse OGD-induced increased astrocyte apoptosis and oxidative stress, and had a protective function against ischemic injury.

The present results showed that OGD up-regulated circ_TTC3 expression and down-regulated miR-138-5p expression of astrocytes, while oleuropein treatments with different concentrations reversed this result in a concentration-reliant manner, suggesting that the protective role of oleuropein in OGD-damaged astrocytes was related to circ_TTC3 and miR-138-5p. Previously, circ_TTC3 has been shown to be highly expressed in astrocytes treated with hypoxia and glucose deficiency, and the loss of circ_TTC3 inhibited the apoptosis and LDH level of astrocytes induced by hypoxia and glucose deficiency and the mechanism was related to sponging miR-372-3p\cite{14}. Circ_TTC3 had distinct elevated level in ischemic myocardium and cardiomyocytes suffering from hypoxic injury, and could absorb miR-15b-5p to isolate and block its liveness to adjust cardiac function after myocardial infarction\cite{34}. In hypoxia-induced HaCaT cells, circ_TTC3 overexpression improved the growth of HaCaT cells and diminished apoptosis by downregulating miR-449a\cite{35}. The results of this experiment were similar to the study of Yang et al\cite{14}, namely, circ_TTC3 silencing could improve the proliferation of astrocytes induced by OGD and inhibit apoptosis and oxidative stress. Besides, miR-138-5p upregulation has been reported to promote the growth and inhibit apoptosis of astrocytes damaged by OGD, along with lessened levels of LDH and inflammatory factors\cite{16}, certifying that miR-138-5p could serve as a potential target in fighting ischemic stroke. In the mechanism study of this experiment, circ_TTC3 targeted miR-138-5p and had an in vs. regulating effect on miR-138-5p level. The effects of oleuropein on astrocyte proliferation, apoptosis and oxidative stress induced by OGD could be reversed by circ_TTC3 overexpression. These results suggested that circ_TTC3 could target miR-138-5p, and the two may be a new mechanism of oleuropein to protect astrocyte damage induced by OGD.

In conclusion, oleuropein inhibited oxidative stress injury of astrocytes induced by OGD through upregulation of circ_TTC3 and inhibiting miR-138-5p, providing new insights into the regulation of oleuropein in ischemic injury.

Conflict of interests:

The authors declared no conflict of interests.

REFERENCES