Chrysins, which targets PLAU, protects PC12 cells from OGD/R-stimulated damage through repressing the NF-κB signaling pathway

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A B S T R A C T

Cerebral ischemia reperfusion injury (CIRI) is a great challenge for the patients with brain ischemia, but its pathophysiological mechanism has not been clearly explored. This study aims to decipher the effect of chrysin and plasminogen activator urokinase (PLAU) in CIRI. The immune-related genes were collected from the ImmPort website, and the differentially expressed genes were determined based on the Gene Expression Omnibus (GEO) database. PC12 cells were used to establish an ischemic stroke model under the condition of oxygen-glucose deprivation and reoxygenation (OGD/R). Small interfering RNA strategy was employed to knock down the PLAU expression of PC12 cells. The proliferation and apoptosis rates of PC12 cells treated by OGD/R or/and chrysin were detected with Cell Counting Kit 8 (CCK-8) and flow cytometry. The protein and mRNA expressions were measured using western blotting and quantitative reverse transcription polymerase chain reaction (qRT-PCR). PLAU was identified as a candidate for CIRI treatment and expressed at higher levels in CIRI tissues compared with that in normal controls. Chrysin was determined as a crucial agent that could decrease the expression of PLAU. Chrysin significantly promoted the cell proliferation, inhibited the protein levels of PLAU, p-NF-κB, and p-IκB in PC12 cells after OGD/R. Silencing of PLAU strengthened the protective effect of chrysin on PC12 cells treated by OGD/R, including the improvement of cell viability and suppression of apoptosis. Chrysin inactivated the NF-κB pathway via targeting PLAU in OGD/R-stimulated PC12 cells. Chrysin prevented PC12 cells from OGD/R-stimulated damage via decreasing PLAU expression and inactivating the NF-κB signaling pathway. © 2022, The Japanese Society for Regenerative Medicine. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Ischemic stroke is considered as a prominent cause of disability and death worldwide [1]. It is an acute cerebrovascular disorder, accounting for more than 80% stroke events induced by cerebral ischemia [2]. Statistical data indicate that a total of 2.5 million people are subjected to the threat of stroke, of which, approximately 1 million will die from this disease or related consequences annually [3,4]. Recently, the timely thrombolysis has been regarded as the most efficacious therapy to ischemic stroke [5].

Unfortunately, this strategy usually induces the brain injury, namely cerebral ischemia reperfusion injury (CIRI) [6]. Existing evidence has suggested that reperfusion could obviously contribute to the recovery of ischemic brain damage and thereby inhibit the development of ischemia stroke [7,8]. However, ischemia/reperfusion (I/R)-induced apoptosis and death of cells could aggravate the brain injury [9]. Hence, it is important to have an in-depth understanding on the molecular mechanism of cerebral ischemia, and seek new efficacious agents as well as underlying therapeutic targets for CIRI.

Bioinformatics screening of this study demonstrated that plasminogen activator urokinase (PLAU) may act as a therapeutic candidate for CIRI. PLAU is also named as urokinase plasminogen activator (uPA) [10]. It can code a serine protease that converts plasminogen to plasmin, facilitating the proteolytic cascade via binding with the relevant receptor. This ability could degrade the extracellular matrix (ECM) components surrounded cancer cells...
[11,12]. Biological processes, including cell migration, invasion, and angiogenesis, are actively regulated by PLAU [12,13]. A previous report has revealed that mutations in PLAU are correlated with Alzheimer’s disease and Quebec Platelet Disorder [14]. Nevertheless, the role of PLAU and its relevant mechanism in CIRI are rarely elucidated.

Chrysin, also known as 5,7-dihydroxyflavone, is a bioactive flavone isolated from honey, propolis, blue passion flower and corresponding pollen [15]. It is widely utilized as herb medicine and has various bioactivities in anti-tumor, antioxidant, antibacterial, and anti-inflammatory aspects [16,17]. Several studies have shown that chrysin is implicated in a wide range of signaling pathways, such as NF-κB, PI3K-AKT, and Wnt-β-catenin, and could regulate cell viability, migration and angiogenesis [18,19]. Importantly, a prior study has indicated that chrysin regulates CIRI in rats through mediating the PI3K/AKT signaling pathway [20]. In the C57BL/J6 mice model, chrysin could protect the brain against cerebral I/R [21]. Our analysis suggested that chrysin maybe function as a putative agent that related with the inhibition of PLAU expression in CIRI. However, there are few investigations about the underlying mechanism of chrysin and PLAU on CIRI. Oxygen-glucose deprivation and reoxygenation (OGD/R)-treated PC12 cells are widely used to mimic CIRI [22]. So, we use OGD/R-treated PC12 cells to unveil the effects of chrysin and PLAU in CIRI.

In this study, we dug into the effect of chrysin on the expression of PLAU and the activity of the NF-κB signaling pathway in PC12 cells treated by OGD/R. We preliminarily proposed a hypothesis that chrysin might have protective potential in OGD/R-stimulated PC12 cells through downregulating PLAU expression and mediating the NF-κB signaling pathway, hoping to provide a novel target for the treatment of CIRI with chrysin.

2. Methods

2.1. Chrysin preparation

Chrysin was purchased from MedChemExpress Company (Monmouth Junction, NJ, USA) and diluted to various concentrations using dimethylsulfoxide (DMSO) and cell culture medium.

2.2. Bioinformatics analysis

Gene Expression Omnibus (GEO) datasets GSE61616 and GSE106680 were exploited to compare the gene expression changes of cerebral ischemia-injured rats before and after treatment with Xuesaitong injection and Danhong injection [24,25]. These two drugs are commonly used in clinical treatment of cerebral infarction or cerebral ischemia in China, and these two data sets are used to screen the targets of effective components of traditional Chinese medicine in treating CIRI. One of the pathological mechanisms of CIRI involves the immune damage caused by acute inflammatory response, and ImmPort database contains immune related genes [26]. After intersecting the genes of GSE61616 and GSE106680 and those from ImmPort database, we obtained 102 genes.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the above 102 genes was carried out by using the online analysis function of DAVID database [27]. Under the condition of FDR<0.05, 38 meaningful pathways were obtained, among which the NF-κB pathway related to CIRI [28] was singled out for gene research. Then, the PLAU gene with the highest logFC value was selected from the genes enriched in NF-κB pathway.

2.3. OGD/R in vitro model

The OGD/R in vitro model was constructed using rat pheochromocytoma PC12 cells (ATCC, USA). The rat pheochromocytoma (PC12) cells were placed in a humidified atmosphere at 37 °C with 5% CO₂, and maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin and 100 U/mL penicillin. To establish the OGD/R model, PC12 cells were first cultured in glucose-free medium and anaerobic chamber (1% O₂, 94% N₂ and 5% CO₂) for 2 hours (h). Then, the glucose-free medium was replaced with DMEM medium containing 10% FBS. Cells were continued to incubate at 37 °C with 5% CO₂ for other 12 h. In control group, PC12 cells were incubated under the normal condition. The different concentrations of chrysin, including 1, 5, 10, 20 and 30 μM, were used to pre-treat PC12 cells, and the treated cells were then subjected to OGD/R.

2.4. Cell transfection

PLAU specific small interfering RNA (si-PLAU, 5’-CCCCTACTATGGCCTGGA-3’) was designed and synthesized by the Genechem to knock down the expression of PLAU in PC12 cells. A scrambled sequence was utilized as the control (si-con, 5’-AAATCTCGAAGCTGACG-3’). According to the protocols of manufacturers, transient transfection was implemented with Lipofectamine 2000 (Invitrogen).

2.5. Cell counting kit 8 (CCK-8) for the detection of cell viability

The viability of PC12 cells was examined using the CCK-8 kit. Cells (1000 cells per well) during the log phase were firstly seeded in a 96-well plate and cultured at 37 °C with 5% CO₂ for 24 h. Next, these cells were stimulated with different concentrations of chrysin and incubated for additional 48 h. Then, 10 μL of CCK-8 cocktail was added into each well for another 1.5-h incubation. The optical density (OD) at a wavelength of 450 nm in each well was used as an indicator for the evaluation of cell viability. For time effect detection, the absorbance was examined every 24 h.

2.6. Flow cytometry for the determination of cell apoptosis

After transfection and OGD/R treatment, PC12 cells were collected in the centrifuged tubes to detect apoptotic ability using an Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium iodide (PI) double staining kit. At 1000 rpm, these cells were centrifuged for 5 minutes (min) and the cell supernatant was then discarded. A total of 500 μL 1 × binding buffer was used to suspend the PC12 cells, and 5 μL of Annexin V-FITC was added into cell suspension to incubate the cells at 37 °C for 5 min in the dark. Subsequently, 5 μL PI and 400 μL PBS were gently mixed and added into the cells, followed by a 5 min-incubation. The apoptotic rate was finally detected with a flow cytometer in line with the manufacturers’ instruction.

2.7. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

PC12 cells were treated with TRizol reagent for the extraction of total RNA. The purity of RNA was measured with the NanoDrop 2000. PrimeScript RT Reagent Kit (Takara, Japan) was applied to convert RNA into complementary DNA (cDNA) on the basis of manufacturer’s guideline. Real-time PCR was carried out with SYBR Premix Ex Taq II (TaKaRa, Japan) on the 7900HT real-time PCR system to measure the expression of PLAU. The recommended
reaction procedures were presented below: initial denaturation at 95 °C for 5 min, and 39 cycles of 30 s at 95 °C, 45 s at 60 °C and 5 min at 72 °C. The expression of PLAU was calculated using 2-ΔΔCT method and normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The forward (F) and reverse (R) primers used in this investigation were listed as follows: PLAU (F: 5'-AGAAGCGACCCTGTGCTATGT -3'; R: 5'-CCACACTGGAGGCTTGTG -3'); GAPDH (F: 5'-TGTGATGGTGAACACAGG -3'; R: 5'-AGTGATGGCATGACGTG -3').

2.8. Western blotting analysis

48 h after transfection, cells were placed into a six-well plate and then exposed to ice-RIPA buffer supplemented with Phenylenediamine (PMSF) for the extraction of protein. Bicinchoninic Acid (BCA) method was utilized to evaluate the concentrations of protein. Following denaturation at 95 °C for 5 min, equal amount (20 μg) of proteins were added into each tank, loaded in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 110 V for 2 h and transferred onto Polyvinylidene fluoride (PVDF) membrane at 90 V for 1.5 h. Afterwards, PVDF membranes were blocked with 5% skimmed milk for 1 h and probed with primary antibodies at 4 °C overnight. Tris Buffered saline Tween (TBST) was employed to rinse membranes three times, each time for 5 min. Next, secondary antibody was utilized to probe PVDF membranes at 37 °C for 1 h. Finally, all protein bands were visualized using enhanced chemiluminescence (ECL) and the intensity was scanned with Quantity One software. GAPDH was considered as an internal control.

2.9. Statistics

All experiments were independently implemented in triplicate. Results were presented as the mean ± standard deviation (SD), which were analyzed by SPSS 22.0 and GraphPad Prism 8.0. The significant difference of two groups was assessed with Student’s t-test, while the comparison of more than two groups was determined using one-way analysis of variance (ANOVA) and Tukey post hoc test. Significant statistical difference was considered when P < 0.05.

3. Results

3.1. A hypothesis proposed: chrysin regulates CIRI through targeting PLAU

To advance the treatment of CIRI, we tried our best to decipher the potential targets. By accessing to ImmPort website and GEO database, we downloaded immune-related genes, and obtained 680 (GSE61616) or 1886 (GSE106680) differential expressed genes. After intersecting with these genes, we obtained a total of 102 common genes (Fig. 1A). Next, the above 102 common genes were analyzed for KEGG pathway enrichment depending on the David database, and a total of 38 meaningful pathways were harvested under the condition of FDR<0.05, including cytokine–cytokine receptor interaction, chemokine signaling pathway, toll-like receptor signaling pathway, NF-κB signaling pathway and Tumor necrosis factor (TNF) signaling pathway (Fig. 1B). These above-mentioned pathways were all related to CIRI. Considering the important effect of NF-κB pathway on CIRI, we selected genes enriched on NF-κB pathway for subsequent analyses, including CD40, PLAU, Bruton’s tyrosine kinase (BTK), Phospholipase C gamma 2 (PLCg2), B-cell linker protein (BLNK), lymphotoxin beta receptor (LTBR), lipopolysaccharide binding protein (LBP), CD14, Toll-like Receptor 4 (TLR4), intercellular adhesion molecule 1 (ICAM1), and TNF receptor super family member 1 A (TNFRSF1A). PLAU with the most significant difference was selected as a putative therapeutic candidate for CIRI. As revealed in Fig. 1C and D, PLAU level was upregulated in CIRI tissues compared with that in normal controls (P < 0.05).

Next, in order to determine which drugs are related with CIRI via targeting PLAU, we visited the Comparative Toxicogenomics Database (CTD) website (https://ctdbase.org/) and ultimately achieved a total of 76 kinds of drugs. Among these drugs, the effect of chrysin on CIRI has been reported [20,29], while little is known about whether the effect of chrysin on attenuating CIRI is related to PLAU. Therefore, we put forward a hypothesis that chrysin might ameliorate the CIRI through decreasing the expression of PLAU. The specific mechanism remains to be elucidated.

3.2. Chrysin suppresses OGD/R-stimulated loss of cell viability and apoptosis

To examine the exact role of chrysin on CIRI, we firstly determined the biological safe doses of chrysin for PC12 cells using CCK-8 assay. CCK-8 results showed that chrysin with diverse concentrations from 1 to 30 μM exerted effects on cell viability, from which chrysin at 10, 20 and 30 μM obviously reduced cell viability in a dose-dependent manner (Fig. 2A, P < 0.05). Subsequently, OGD/R-treated PC12 cells were exposed to biological safe concentrations of chrysin (1, 5, 10, 20, and 30 μM). As illustrated in Fig. 2B, a significant loss of PC12 cell viability was induced in OGD/R group relative to that in the control group (P < 0.05). Elevations in cell viability were found in 5, 10, 20, and 30 μM chrysin groups, and the most improvement was observed at 20 μM (Fig. 2B, P < 0.05). Thus, we selected 20 μM of chrysin for the following experiments. To detect the time effect of chrysin on the viability of OGD/R-treated PC12 cells, CCK-8 assay was conducted to measure cell proliferation at 0 h, 24 h, 48 h and 72 h after OGD/R or/and chrysin treatments. Fig. 2C manifested that chrysin significantly attenuated the OGD/R-stimulated viability of PC12 cells in a time-dependent manner (P < 0.05). Increased apoptosis induced by OGD/R was dwindled after chrysin treatment (Fig. 2D, P < 0.01). Collectively, these data illuminated that chrysin could attenuate the loss of viability and apoptosis of OGD/R-treated PC12 cells.

3.3. Chrysin attenuates the PLAU expression and inactivates NF-κB pathway in OGD/R-stimulated PC12 cells

Based on the bioinformatics analysis, chrysin was found to be related with the downregulation of PLAU, and PLAU was observed to be enriched in NF-κB signaling pathway. Herein, we performed Western blot to measure the protein levels of PLAU and NF-κB pathway-related markers in OGD/R or/and chrysin-treated PC12 cells (Fig. 3, P < 0.01). By contrast to the control group, OGD/R group appeared increased protein levels of PLAU, phospho (p)-NF-κB, and p-IκB. However, this tendency was reversed by chrysin (Fig. 3, P < 0.01). These results manifested that chrysin inhibited the expression of PLAU and inactivated the NF-κB signaling pathway in OGD/R-treated PC12 cells.

3.4. Chrysin relieves the OGD/R-stimulated injury of PC12 cells via downregulating the expression of PLAU

To further clarify the underlying mechanism between chrysin and PLAU in CIRI, we then used si-PLAU to downregulate the PLAU expression of PC12 cells. As expected, qRT-PCR and Western blot experiments denoted that PLAU expression was decreased in PC12 cells (Fig. 4A and B, P < 0.01). By contrast to the OGD/R, the interference of si-PLAU significantly promoted cell proliferation of
Fig. 1. PLAU was expressed in cerebral ischemia reperfusion injury (CIRI) tissues and related with the NF-κB pathway. (A) Potential therapeutic candidates were predicted in three bioinformatics datasets. (B) A total of common genes were analyzed for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment through accessing to the David database. (C–D) The expression of plasminogen activator urokinase (PLAU) was determined in CIRI tissues and normal controls, based on datasets GSE61616 and GSE106680. *P* < 0.05 compared with controls.

Fig. 2. Chrysin repressed oxygen-glucose deprivation and reoxygenation (OGD/R)-stimulated loss of cell viability and apoptosis. (A) Cell Counting Kit 8 (CCK-8) assay uncovered that chrysin (1, 5, 10, 20, and 30 μM) was safe for PC12 cells. *P* < 0.05 and **P < 0.01, compared with control group. Chrysin inhibited OGD/R-induced PC12 cell viability in a dose-dependent manner (B) and in a time-dependent manner (C). *P < 0.05 and **P < 0.01, compared with control group; *P < 0.05 and ***P < 0.01, compared with OGD/R group. (D) AnnexinV FITC analysis. **P < 0.01, compared with control group; ***P < 0.01, compared with OGD/R group. Quantified data are presented as mean ± standard deviation (SD), and were analyzed by one-way analysis of variance (ANOVA) and Tukey comparison test (n = 3).
OGD/R-treated PC12 cells, which was consistent with the effect of chrysin. Apart from this, the combination of chrysin and si-PLAU further elevated cell viability compared with OGD/R + chrysin or OGD/R + si-PLAU (Fig. 5B, \( P < 0.05 \)). Conversely, flow cytometric analysis revealed that the apoptotic rate of OGD/R-induced PC12 cells was remarkably reduced because of chrysin treatment or si-PLAU transfection. The apoptosis ability of OGD/R-treated PC12 cells was further inhibited after chrysin treatment and si-PLAU transfection (Fig. 5A and C, \( P < 0.01 \)). All results suggested that chrysin induced the promotion of OGD/R-treated PC12 cells viability and suppression of apoptosis rate through inhibiting the expression of PLAU.

3.5. PLAU is involved in the regulatory action of chrysin to the inactivation of NF-κB pathway

To explore whether PLAU is implicated in the regulatory action of chrysin to the activity of the NF-κB pathway, western blotting experiment was then conducted to measure the protein levels of p-NF-κB/NF-κB and p-IκB/IκB in OGD/R, OGD/R + chrysin, OGD/R + si-PLAU, and OGD/R + chrysin + si-PLAU groups (Fig. 6, \( P < 0.01 \)). Compared with OGD/R, chrysin treatment and si-PLAU transfection all reduced the expression of PLAU, respectively. Additionally, the protein level of PLAU was further suppressed due to the combination of chrysin treatment and si-PLAU transfection. Likely, the protein levels of p-NF-κB/p-IκB were all dramatically decreased in OGD/R + chrysin + si-PLAU group compared with those in OGD/R + chrysin or OGD/R + si-PLAU group. These findings above illustrated that PLAU was involved in the regulatory action of chrysin to the inactivation of the NF-κB pathway.

4. Discussion

Currently, accumulating evidence has illustrated that the immune injury caused by acute inflammation plays an increasingly important role in cerebral ischemia [30]. After cerebral ischemia, cell damage will be accelerated due to the overexpression of multiple cytokines and infiltration of inflammatory cells [31,32]. Therefore, to decipher the novel potential therapeutic targets for CIRI, we conducted bioinformatics analysis and processed relevant data. Finally, on the basis of previous publications and logFC ranking, PLAU enriched in the NF-κB signaling pathway was identified as a promising therapeutic element for CIRI. There was a report that the depletion of PLAU expression modulated by miR-193a-3p could block tumor cell proliferation and hinder the progression of colorectal cancer [10]. Knockdown of PLAU evidently triggers the increase of apoptosis via the DNA-damage pathway [33]. The American Society Clinical Oncology (ASCO) has determined that PLAU can function as a potential therapeutic factor for risk assessment, and the upregulation of PLAU is associated with unfavorable prognosis [34]. Taken together, the crucial effects of PLAU in tumorigenesis and angiogenesis have been well characterized. However, the specific role of PLAU in CIRI has been yet detected. Our data revealed that PLAU expression was remarkably increased in CIRI tissues relative to that in normal controls, which suggested that the mutation of PLAU may affect the progression of CIRI. Next, CTD website was utilized to seek the possible drugs that correlated with the declined expression of PLAU in CIRI. Ultimately, chrysin was singled out. Chrysin is widely distributed in plants with relatively low toxicity, so it is considered as an important resource in agent development. Emerging publications...
have reported that chrysin exerts anti-apoptotic and anti-inflammatory activities, which can not only attenuate the oxidative stress, but also block the development of cancers [35–37]. In several prior researches, the neuroprotective impact of chrysin in OGD/R-stimulated cells has been determined through regulating cellular processes and essential signaling pathways [20,21]. Consistent with the reported neuroprotective impact, this present research demonstrated that chrysin markedly promoted cell viability of OGD/R-treated PC12 cells in the dose-/time-dependent manners. Furthermore, silencing of PLAU further strengthened the neuroprotective effect of chrysin on the OGD/R-treated PC12 cells.

After I/R, cells of brain tissues are regulated by multiple inflammatory cytokines, such as TNF-α, Interleukin (IL)-1β and IL-6 [38]. NF-κB signaling pathway is implicated in the transcription of mass of inflammatory factors and apoptosis-related genes, and regulates inflammation cascades and apoptosis [39]. NF-κB is reported to participate in the acute phase of cerebral ischemia and its expression is rapidly elevated after cerebral ischemia [20,40]. The current investigation has manifested that the infarct volume can be significantly reduced via inactivated NF-κB signaling pathway, which discloses that the inactivation of NF-κB pathway possesses a neuroprotective effect on cells after cerebral ischemia. IkB is an upstream factor associated with the activation of NF-κB pathway.
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[41]. Based on our results of this work, PLAU was a potential target gene enriched in NF-κB pathway, and chrysirn treatment induced the suppression of PLAU expression as well as p-NF-κB and p-IκBα levels. These phenomena were consistent with the viewpoints that have certificated in a previous study [20]. Taken together, these findings unearied that chrysirn may protect PC12 cells from OGD/R-induced injury by decreasing the PLAU expression and inactivating the NF-κB pathway.

5. Conclusion

In conclusion, chrysin exerts a protective role in OGD/R-treated PC12 cells by inhibiting PLAU expression and inactivating of NF-κB signaling pathway. Collectively, chrysirn, which targets PLAU, might function as a promising therapeutic candidate for cerebral ischemia, and its function is worth to be further explored in vivo.

Authors’ contributions

NL and WXZ conceived the project and participated in the study design, supervision of laboratory processes analysis, and interpretation of the results. NL participated in drafting the manuscript. YL, and JRL helped in protein experiments and data analysis. WXZ provided the critical review in the manuscript preparation. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated for this study are available on request to the corresponding author.

Ethics approval and consent to participate

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Declaration of competing interest

All authors declare that they have no conflict of interests.

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References

[1] Ou J, Kou L, Liang L, Tang C. MR-375 attenuates injury of cerebral ischemia/reperfusion via targeting Ctgf. Biosci Rep 2017;37.
[2] Campbell BC, Mitchell PJ, Kleing Tj, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 2015;372:1009–18.
[3] Li F, Shi W, Zhao EY, Geng X, Li X, Peng C, et al. Enhanced apoptosis from early physical exercise rehabilitation following ischemic stroke. J Neurosci Res 2017;95:1017–24.
[4] Zuo XL, Deng HL, Wu P, Xu E. Do different reperfusion methods affect the outcomes of stroke induced by MCAO in adult rats? Int J Neurosci 2016;126:850–5.
[5] Blakeley JO, Ulinas RH. Thrombolytic therapy for acute ischemic stroke. J Neurol Sci 2007;261:55–62.
[6] Zhai WW, Sun L, Yu ZQ, Chen G. Hyperbaric oxygen therapy in experimental and clinical stroke 2016;6:111–8.
[7] Fluri F, Schuhmann MK, Kleinschmitz C. Animal models of ischemic stroke and their application in clinical research. Drug Dev Res Ther 2015;5:344–54.
[8] Xing R, Chen H, Zhang D, Jiang R, Liu X, et al. Ischemic postconditioning inhibits apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Stroke 2008;39:2362–9.
[9] Jiang D, Sun X, Wang S, Man H. Upregulation of miR-874-3p decreases cerebral ischemia/reperfusion injury by directly targeting BMP and BCL2L13. Biomed Pharmacother 2019;117:108941.
[10] Lin M, Zhang Z, Gao M, Yu H, Sheng H, Huang J. MicroRNA-193a-3p suppresses the colorectal cancer cell proliferation and progression through down-regulating the PLAU expression. Cancer Manag Res 2019;11:935–41.
[11] Montuori N, Pesapane A, Rossi FW, Giudice V, De Paulis A, Selleri C, et al. Urokinase type plasminogen activator receptor (uPAR) as a new therapeutic target in cancer. Transl Med Unisfa 2016;15:15–21.
[12] Mahmood N, Mikhailou C, Rabbani SA. Multilactated role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): diagnostic, prognostic, and therapeutic applications. Front Oncol 2018;8:24.
[13] Mauro CD, Pesapane A, Formisano L, Rosa D, Annato V, Ciciola P, et al. Urokinase-type plasminogen activator receptor (uPAR) expression enhances invasion and metastasis in RAS mutated tumors 2017;7:9388.
[14] Riemenschnieder M, Kanta L, Friedrich P, Schwarz S, Taddei K, Neff F, et al. A functional polymorphism within plasminogen activator urokinase (PLAU) is associated with Alzheimers’s disease. Hum Mol Genet 2006;15:2446–56.
[15] Lirdprapampokkhol K, Sakurai H, Abdelhamel S, Yokoyama S, Maruyama T, Athikomkalchial S, et al. A flavonoid chrysin suppresses hypoxic survival and metastatic growth of mouse breast cancer cells. Oncol Rep 2013;20:2357–64.
[16] Du Q, Yin X, Cai J, Huang M, Su M, Chui H. Chrysin attenuates cerebral ischemia/reperfusion injury by modulating the transcription factors T-bet and GATA-3 in mice. Mol Med Rep 2012;6:100–4.
[17] Parajuli P, Joshee N, Rimedo AM, Mittal S, Yadav AK. In vitro antitumor mechanisms of various Scutellaria extracts and constituent flavonoids. Planta Med 2005;71:41–8.
[18] Lee SJ, Yoon JT, Song KS. Chrysin inhibited stem cell factor (SCF)/c-Kit complex-induced cell proliferation in human myeloid leukemia cells. Biochem Pharmacol 2007;74:215–25.
[19] Gao AM, Ke ZP, Shi F, Sun GC, Chen H. Chrysin enhances sensitivity of BEL-7402/ADM cells to doxorubicin by suppressing PI3K/Akt/Erk2 and ERK/Erk pathway. Chem Biol Interact 2013;206:100–8.
[20] Li TF, Ma J, Han XW, Jia XY, Yuan HF, Shi SF, et al. Chrysin ameliorates cerebral ischemia/reperfusion (I/R) injury in rats by regulating the PI3K/Akt/mTOR pathway. Neurochem Int 2019;129:104496.
[21] Du F, Zou T, Ozturk MN, Basil Turkmen N, Cigdem O, Tajdade A, Tecellolju M, et al. Chrysin prevents brain damage caused by global cerebroischemia/reperfusion in a C57Bl/6 mice model. Turk J Med Sci 2016;46:1926–33.
[22] Wen Z, Hou W, Wu W, Zhao Y, Dong X, Bai X, et al. G-O-Galloylpedorforinol attenuates cerebral ischemia/reperfusion-induced neuroinflammation and oxidative stress via PI3K/Akt/Nrf2 activation. Oxid Med Cell Longev 2018;2018:8678267.
[23] Zhang Y, Zhang Y. IncRNA ZFAS1 improves neuronal injury and inhibits inflammation, oxidative stress, and apoptosis by sponging miR-582 and upregulating NO3S expression in cerebral ischemia/reperfusion injury. Inflammation 2020;43:1337–50.
[24] Qian J, Zhao X, Wang W, Zhang S, Hong Z, Chen X, et al. Transcriptomic study reveals recovery of impaired astrocytes contribute to neuroprotective effects of Danhong injection against cerebral ischemia/reperfusion-induced injury. Front Pharmacol 2018;9:250.
[25] Wang L, Yu Y, Yang J, Zhao X, Li Z. Dissecting Xuesaotang’s mechanisms on preventing stroke based on the microarray and connectivity map. Mol Biosyst 2015;11:3033–9.
[26] Bhatthacharya S, Dunn P, Thomas CG, Smith B, Schaefer H, Chen J, et al. Immunohort, toward repurposing of open access immunological assay data for translational and clinical research. Sci Data 2015;2:180015.
[27] Dennis Jr G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003;4:93.
[28] Xue X, Wu J, Zhao X, Li Z. Prognosis and predictive values associated with Alzheimer’s disease. Hum Mol Genet 2006;15:2446–56.
[29] Bellocq JP, Luporsi E, Barri

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Deldar Y, Pilehvar-Soltanahmadi Y, Dadashpour M, Montazer Saheb S, Rahmati-Yamchi M, Zarghami N. An in vitro examination of the antioxidant, cytoprotective and anti-inflammatory properties of chrysin-loaded nanofibrous mats for potential wound healing applications. Artif Cells Nanomed Biotechnol 2018;46:706–16.

Ramírez-Espinosa JJ, Saldaña-Ríos J, García-Jiménez S, Villalobos-Molina R, Ávila-Villarreal G, Rodríguez-Ocampo AN, et al. Chrysin induces anti-diabetic, antidyslipidemic and anti-inflammatory effects in athymic nude diabetic mice, vol. 23; 2017.

Roy S, Sil A, Chakraborty T. Potentiating apoptosis and modulation of p53, Bcl2, and Bax by a novel chrysin ruthenium complex for effective chemotherapeutic efficacy against breast cancer. J Cell Physiol 2019;234:4888–909.

Zheng T, Jiang H, Jin R, Zhao Y, Bai Y, Xu H, et al. Ginsenoside Rg1 attenuates protein aggregation and inflammatory response following cerebral ischemia and reperfusion injury. Eur J Pharmacol 2019;853:65–73.

Sui X. Inhibition of the NF-κB signaling pathway on endothelial cell function and angiogenesis in mice with acute cerebral infarction. J Biol Regul Homeost Agents 2019;33:375–84.

Zhao Y, Wang H, Chen W, Chen L, Liu D, Wang X, et al. Melatonin attenuates white matter damage after focal brain ischemia in rats by regulating the TLR4/NF-κB pathway. Brain Res Bull 2019;150:168–78.

Sundarraj K, Raghunath A, Panneerselvam L, Perumal E. Fisetin, a phytophenol, targets apoptotic and necroptotic cell death in HepG2 cells 2020;46:118–35.