



Salidroside-pretreated mesenchymal stem cells contribute to neuroprotection in cerebral ischemic injury in vitro and in vivo

Liping Zhou¹ · Panpan Yao¹ · Lixia Jiang¹ · Zhaoyun Wang¹ · Xiaohe Ma¹ · Guangxin Wen¹ · Jintao Yang¹ · Binjie Zhou¹ · Qin Yu¹

Received: 28 August 2020 / Accepted: 23 September 2021 / Published online: 27 September 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract

Mesenchymal stem cells (MSCs) are considered a promising tool for treating cerebral ischemic injury. However, their poor survival after transplantation limits their therapeutic effect and applications. Salidroside has been reported to exert potent cytoprotective and neuroprotective effects. This study aimed to investigate whether salidroside could improve MSC survival under hypoxic-ischemic conditions and, subsequently, alleviate cerebral ischemic injury in a rat model. MSCs were pretreated by salidroside under hypoxic-ischemic conditions. The cell proliferation, migratory capacity, and apoptosis were evaluated by means of Cell Counting Kit-8, transwell assay, and flow cytometry. MSCs pretreated with salidroside were transplanted into the rats subsequent to middle cerebral artery occlusion. The grip strength, 2,3,5-triphenyltetrazolium chloride, and hematoxylin–eosin staining were used to analyze the therapeutic efficiency and pathological changes. The mature neuron marker NeuN and astrocyte marker GFAP in the focal area were detected by immunofluorescence. These results indicated that salidroside promoted the proliferation, migration and reduced apoptosis of MSCs under hypoxic-ischemic conditions. In vivo experiments revealed that transplantation of salidroside-pretreated MSCs strengthened the therapeutic efficiency by enhancing neurogenesis and inhibiting neuroinflammation in the hippocampal CA1 area after ischemia. Our results suggest that pretreatment with salidroside could be an effective strategy to enhance the cell survival rate and the therapeutic effect of MSCs in treating cerebral ischemic injury.

Keywords Mesenchymal stem cells · Salidroside · Cerebral ischemic injury · Cell survival · Neuroprotection

Introduction

Brain hypoxia–ischemia has been considered a critical factor in many human central nervous system diseases, including stroke, which is the leading cause of disability and the second most common cause of mortality worldwide (Chen et al. 2019; Mangin et al. 2019). Although progress has been made on fundamental research and clinical treatment, there are no efficient therapies to ameliorate the resulting neurodegeneration.

Mesenchymal stem cells (MSCs) have displayed capabilities in regenerative therapy for the treatment of stroke because of their multi-directional differentiation potential and hypo-immunogenicity properties (Zomer et al. 2015). Transplantation of MSCs enhances functional recovery and endogenous neurogenesis in stroke models (Cunningham et al. 2018; Zhang et al. 2015). The cell survival rate after MSCs are transplanted into the ischemic region is quite low, which has limited the application of MSCs (Feng et al. 2016; Hedhli et al. 2017; Zhang et al. 2018). Hence, promoting cell survival and migration potential of the MSCs is one of the critical issues for stem cell-based therapy for cerebral ischemic injury.

Salidroside (p-hydroxyphenethyl- β -D-glucoside, Sal), a phenylpropanoid glycoside isolated from the *Rhodiola rosea*, has been widely used as a traditional herbal medicine that exhibits potent anti-inflammatory and anti-apoptotic functions (Bai et al. 2014). Previous studies have shown that salidroside could promote the proliferation of MSCs

Liping Zhou and Panpan Yao have contributed equally to this work.

✉ Qin Yu
qinyu3587@126.com

¹ College of Life Science, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, People's Republic of China

(Zhao et al. 2014b) and induce MSCs to differentiate into cholinergic neurons (Zhang et al. 2012) and dopaminergic neurons (Zhao et al. 2014a). Concurrently, salidroside has been reported to improve brain ischemic injury by reducing neuroinflammation and neural damage (Zhang et al. 2019; Zhong et al. 2018; Zuo et al. 2018).

In the present study, we therefore investigated the effect of salidroside on survival and migration potential of MSCs under hypoxia–ischemia and focused on the therapeutic effect by which salidroside-pretreated MSCs induce neuro-protective effects following cerebral ischemic injury.

Methods

Animals

In the present study, male Sprague–Dawley (SD) rats (80–120 g) were used for MSC isolation and culture. SD rats (260–300 g) were used for Middle cerebral artery occlusion (MCAO) model establishment. All rats were provided by Zhejiang Chinese Medical University Animal Center (Laboratory Animal Certificate: SYXK 2013-0184). Rats were raised in individual cages in a temperature- and humidity-controlled room with a 12-h light/dark cycle and access to food and water ad libitum.

MSC culture and pretreatment

MSCs were isolated from SD rats (80–120 g) under sterile environment as previously reported (Ge et al. 2016). The long bone was taken from the hind legs of SD rats, and both ends of the long bones were cut. Bone marrow was flushed out with phosphate buffered saline (PBS, BOSTER, China). The obtained bone marrow solution was centrifuged at 1000 rpm for 5 min, the supernatant liquid was removed, and the cell pellets were resuspended in Dulbecco's Modified Eagle's Medium/Ham's F-12 (DMEM/F12, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA). Cells were detached with 0.25% trypsin-ethylene diamine tetraacetic acid (EDTA, Gibco, USA) at 80% confluence. Passage three cells were used for the following experiments.

For hypoxia and ischemia, MSCs were pretreated with serum-free medium with 100 $\mu\text{mol/l}$ of Cobalt chloride (CoCl_2 , Sigma, USA) for 12 h. For salidroside (HPLC $\geq 98\%$, Shanghai Yuanye Bio-Technology Co., Ltd., China) pretreatment, MSCs were cultured with different concentrations of salidroside (0.375, 0.75, 1.5, 3, 6 $\mu\text{g/ml}$) for 48 h, followed by exposure to 100 $\mu\text{mol/l}$ of CoCl_2 with serum-free medium for 12 h. The MSCs cultured in complete medium served as control. These pretreated cells

were used for Cell Counting Kit (CCK)-8 assay, transwell assay, and flow cytometry assay.

CM-Dil (Invitrogen, Carlsbad, CA, USA) was used to label the cell membrane prior to transplantation, without affecting cell morphology, viability, and proliferation capacity. CM-Dil stock solution was prepared as recommended by the manufacturer.

CCK-8 assay

MSC proliferation was measured using the CCK-8 (Dojindo Laboratories, Inc., Kumamoto, Japan). MSCs were inoculated at 1×10^3 cells/well in 96-well culture plates. The medium was discarded, and each well was washed twice with PBS. One hundred microliters of DMEM/F12 and 10 μl of CCK-8 reagents were added to each well and incubated at 37 $^\circ\text{C}$ with 5% CO_2 for 2 h. The absorbance of the reaction solution was measured at 450 nm using a microplate reader (SpectraMax Plus384, Molecular Devices, LLC, Sunnyvale, CA, USA).

Transwell assay

Cell migration ability was performed using transwell plates (24-well plate, 8- μm pore size, Corning, NY, USA). Cells were digested and resuspended in DMEM/F12 containing 2% FBS. One hundred fifty microliters (1×10^5 cells/ml) of cell suspension was added to the upper chamber of the migration well, and 600 μl of complete medium was added to the lower chamber. After 15 h, cells from the top of the filter were carefully removed with a cotton swab. The cells that had migrated through to the underside of the insert membranes were then fixed with 4% paraformaldehyde for 30 min and stained with crystal violet for 10 min. Migrated cells were observed and imaged by a fluorescence microscope (Nikon, Eclipse Ts2-FL, Japan). The number of migrated cells was determined manually in six random fields per well by six independent, blinded investigators.

Flow cytometry assay

To quantify the apoptotic MSCs, cells were collected, washed with PBS, and then centrifuged at 1000 rpm for 5 min before 1×10^5 cells were resuspended in the $1 \times$ AnnexinV binding buffer mixed with 5 μl of propidium iodide (PI) reagent. After incubation for 15 min at room temperature in the dark, another 400 μl of binding buffer was added, and cells were measured using a flow cytometry machine (Accuri C6, BD Biosciences).

MCAO model

MCAO was induced by the modified Zea Longa method (Jiang et al. 2018). Rats were anesthetized with 10% chloral hydrate (0.35 ml/100 g, Yonghua Chemical, China) and fixed on an operating table in a supine position. A midline neck incision was made, and the Common carotid artery (CCA) and its bifurcation were exposed and isolated carefully from the vagus nerve. The bifurcation of the external common carotid artery and the proximal end of CCA were occluded by a silk suture. A small hole was cut into the CCA to insert a filament into the internal CCA. The suture was tightly tied around the filament to prevent bleeding and the reverse-action tweezers were removed. After 1.5 h of occlusion, the suture was withdrawn to enable reperfusion and the wound and skin were sutured. Finally, animals were placed in a cage over a warming blanket until full recovery from anesthesia. Rats in sham group underwent the same procedure, but the filament was not inserted.

Transplantation of MSCs and animal grouping

Sixty rats were randomly divided into four groups ($n = 15$ per group), including the sham, vehicle, MSCs (MSCs transplantation), and Sal-MSCs (cells pretreated with 0.75 $\mu\text{g/ml}$ salidroside). Three days after MCAO, the anesthetized rats were fixed on a stereotaxic brain locator. For the MSC and Sal-MSC groups, 10 μl of cells (1×10^7 cells/ml) were engrafted at 3.6 mm toward the caudal side of the frontal fontanel and at 2 mm on the right side of the midline. The depth of the injection was 3 mm, and the rate of the injection was 1 $\mu\text{l}/\text{min}$. Rats of the sham and vehicle groups were transplanted with the same volume of PBS. Behavioral testing ($n = 3$ per group) was performed at 7, 14, and 21 days after MCAO. The rats were euthanized 14 days after MCAO to obtain the brain tissue samples for 2,3,5-triphenyltetrazolium chloride (TTC) staining ($n = 3$ per group), hematoxylin–eosin (HE) staining ($n = 3$ per group), and immunofluorescence ($n = 6$ per group). For each group, the sections are from different rats.

Behavioral test

Grip strength was measured at 7, 14, and 21 days after MCAO as previously described (Pin-Barre et al. 2014). Briefly, each rat was held by its tail and gripped the tension bar of the instrument using their limbs. Once the grip was secured, the animal was slowly pulled away from the bar. A digital reading (in Newtons) of three successive trials was obtained for each rat, and then averaged for analysis.

TTC staining

To measure infarct volumes, rats were decapitated at 14 days after MCAO. Brains were cut into 2-mm-thick coronal sections and incubated in 2% TTC (Sigma, USA) solution for 30 min at 37 °C in the dark. Infarct volumes were determined by the ImagePro Plus 6.0 software and expressed as the percentage of the total volume of the cerebral tissue.

Preparation of paraffin section

Brains from anesthetized rats were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde. Then, brains were removed, post-fixed, dehydrated in graded alcohol, and embedded in paraffin. Coronal sections at 4 μm were used for HE staining and immunofluorescence.

HE staining

Paraffin sections were subjected to conventional dewaxing, hydration, hematoxylin staining, and hydrochloric acid ethanol differentiation. After rinsing with distilled water, the sections were stained with 1% eosin solution (BOSTER, China) for 1 min with subsequent dehydration in graded alcohol; the sections were dewaxed in xylene and mounted with neutral gum. The mounted slides were observed and imaged by microscope.

Immunofluorescence

CM-Dil-labeled MSCs were observed by fluorescence microscopy to detect the implanted cells in the host brain. Neuronal nuclear antigen (NeuN) and glial fibrillary acidic protein (GFAP) expression were assessed by evaluating the number of NeuN- and GFAP-positive cells in the ischemic boundary zone. Sections were dewaxed, hydrated, repaired by citric acid antigen, and incubated with 3% H_2O_2 for 10 min at room temperature. Then, sections were incubated with rabbit anti-NeuN antibody (1:5,000, Abcam) and rabbit anti-GFAP antibody (1:5,000, Abcam) overnight at 4 °C and incubated with a secondary antibody at 37 °C for 30 min. Whole sections were counter-stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma, USA). Ten fields of each section were randomly imaged at high magnification (400 \times) to count the CM-Dil-labeled MSCs NeuN- and GFAP-positive cells. Positive cells were counted by ImagePro Plus 6.0.

Statistical analysis

All experimental data are expressed as the mean \pm standard deviation. The differences between multiple groups were analyzed by one-way analysis of variance (ANOVA).

Comparisons between two groups were performed via the Dunnett's t-test. All were considered statistically significant for $P < 0.05$.

Results

Salidroside promoted MSC proliferation

MSCs were cultured and passed until passage 3. The cell morphology tends to be uniform, showing a spindle shape with high uniformity. To determine the influence of salidroside on MSC proliferation, the number of MSCs was measured using the CCK-8 test. As shown in Fig. 1a, the proliferation rate of MSCs within hypoxic-ischemic treatment was significantly reduced. In addition, there was an increase in the MSCs treated by different concentrations of salidroside (0.375, 0.75, 1.5, 3, 6 $\mu\text{g/ml}$) (Fig. 1a). These results indicated that salidroside promotes cell proliferation under hypoxic-ischemic conditions.

Salidroside improved MSC migration

Transwell assay was performed to determine the migration potential of MSCs, and the migrated cells were detected (Fig. 1b). Compared to normoxic conditions, the migration

of MSCs are suppressed under hypoxic-ischemic conditions. Concomitantly, we found that MSCs migrated to the lower compartment more rapidly in response to the salidroside (0.375, 0.75, 1.5, 3, 6 $\mu\text{g/ml}$), especially in the 0.75 $\mu\text{g/ml}$ of salidroside treatment (Fig. 1c). These results confirmed that salidroside promoted the migration of MSCs against hypoxia–ischemia.

Salidroside protected MSCs from apoptosis

We analyzed the effect of salidroside pretreatment on the apoptosis rate of MSCs. Data revealed that exposure of MSCs to hypoxic-ischemic conditions induced MSCs apoptosis, whereas salidroside decreased the percentage of apoptotic cells, especially in the 0.75 $\mu\text{g/ml}$ of salidroside treatment (Fig. 2). Such findings suggested that the number of MSCs which were apoptotic upon treatment with hypoxia–ischemia were restored by salidroside pretreatment.

Salidroside-pretreated MSCs ameliorated brain injuries in MCAO rats

To evaluate the therapeutic effects of Sal-MSCs, the cells were labeled with CM-Dil, a fluorescence marker in vivo (Fig. 3), and then 1×10^5 MSCs or Sal-MSCs were transplanted into the ischemic boundary zone 3 days after MCAO.

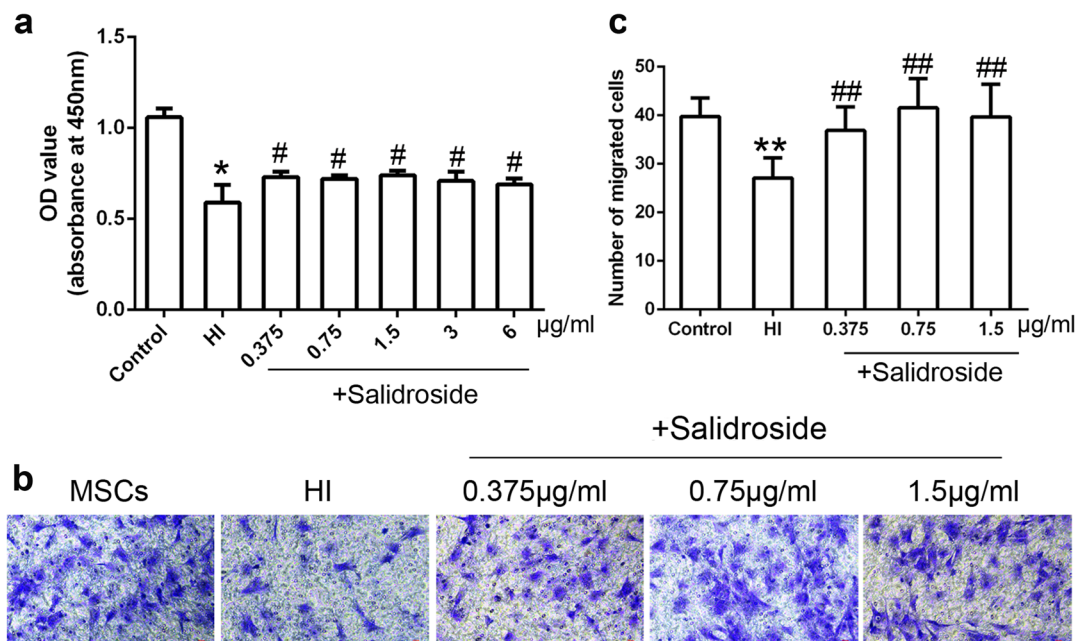


Fig. 1 The proliferative ability and migratory capacity of MSCs were promoted by salidroside under hypoxia–ischemia. Cell proliferation was detected by Cell Counting Kit-8 (**a**). Cell migration was examined by transwell assay. Migrated cells were stained with crystal violet. The number of migrated cells was determined manually

in 6 random fields per well by six independent, blinded investigators (**b**). The quantification of migrated MSCs under different concentrations (0.375, 0.75, and 1.5 $\mu\text{g/ml}$) of salidroside (**c**). * $P < 0.05$ and ** $P < 0.01$ versus control group, # $P < 0.05$ and ## $P < 0.01$ versus vehicle group

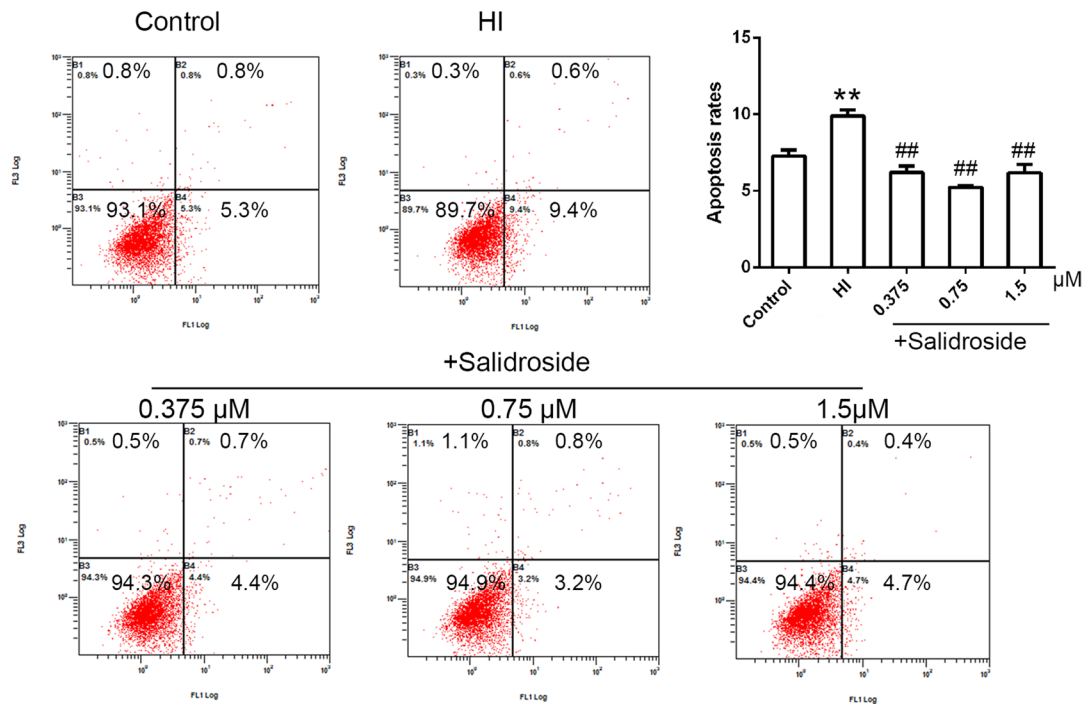


Fig. 2 The apoptosis rate of MSCs under hypoxic-ischemic conditions was reduced by salidroside. Quadrant graphs of Annexin V (FL1)/PI (FL3) double staining with flow cytometer. The lower left quadrant represented the live cells, the lower right quadrant represented the early apoptotic cells. Numbers on each individual plot

refers to the percentage positive cells. Data represent one of three different experiments. Data are presented as histogram chart representing three independent experiments with mean \pm SD. ** $P < 0.01$ versus control group, ## $P < 0.01$ versus vehicle group

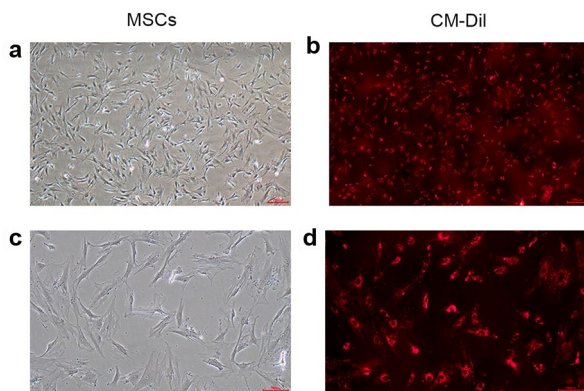


Fig. 3 MSCs were labeled with CM-Dil in vitro. MSCs from passage three were observed under the inverted fluorescence microscope (a, c). The phase-contrast and fluorescent image of CM-Dil labeled MSCs (red) (b, d)

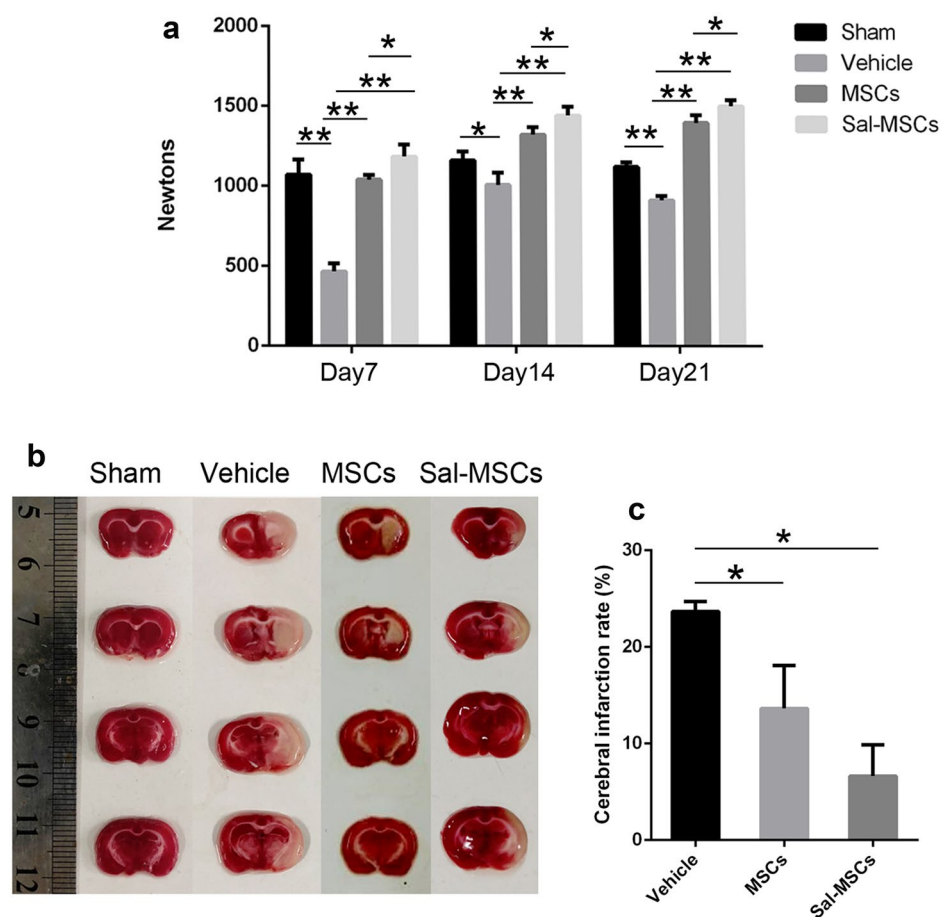
The grip strength was investigated in the sham, vehicle, and Sal-MSC groups, respectively, on days 7, 14, and 21. Deficits in grip strength reached their lowest point in ischemic injury rats, whereas the grip force of Sal-MSCs group was significantly elevated compared to the rats in MSC group (Fig. 4a). We compared the infarction areas in coronal sections from animals of the vehicle, MSC, and Sal-MSC

groups on day 14 (Fig. 4b). TTC staining was used to assess lesion volume as a percentage of contralateral hemispheric volume. Fourteen days after MCAO, significant differences of reduction in infarct volume were detected in the Sal-MSC and MSC groups compared with the vehicle group (Fig. 4c).

Salidroside-pretreated MSCs alleviated pathological changes in the hippocampal CA1 area after ischemia

After transplantation of MSCs was accepted, CM-Dil labeled MSCs were found in the hippocampal CA1 area of rat brains (Fig. 5a). In the sham and vehicle groups, MSCs were not transplanted. After transplantation of MSCs, the number of CM-Dil positive cells in the Sal-MSCs group were significantly higher than that in the MSCs group (Fig. 5b). HE staining provided an objective assessment on pathological changes (Fig. 5c). Examinations of the hippocampus showed that the morphological structures of the hippocampus in the sham group were normal, with uniform dyeing. By contrast, data showed that degeneration and necrosis of nerve cells in hippocampal formations were aggravated in rats that suffered from ischemia. Similar but slight pathological changes were observed in the MSC group subsequent to ischemia; however, the changes in the MSCs with salidroside pretreatment were less marked than those of the vehicle group. This

Fig. 4 Salidroside-pretreated MSCs ameliorated brain injuries in MCAO rats. Grip strength for rats in sham group and subject to cerebral ischemic injury (a). Brain slices were stained with TTC to visualize lesions (b). Quantitative analysis of infarction volume (c). * $P < 0.05$, ** $P < 0.01$



observation suggested that the transplantation of MSCs ameliorated pathological changes after cerebral ischemic injury, while salidroside pretreatment could strengthen therapeutic efficiency.

Salidroside-pretreated MSCs enhanced neurogenesis in the ischemic hippocampus

To investigate whether these transplanted MSCs could differentiate into mature neurons, CM-Dil labeling of the cells with the mature neuron marker NeuN was performed. Figure 6a shows that many neurons were positive in the hippocampal CA1 area. However, only a few CM-Dil-positive cells co-labeled with NeuN were observed in the MSCs group. As expected, there were more double-labeled cells in the Sal-MSCs group (Fig. 6b). These data provided evidence that transplantation of Sal-MSCs enhanced neurogenesis to a greater extent than transplantation of MSCs alone.

In addition, cells with astrocyte marker GFAP were counted in the same area (Fig. 7a). The GFAP-positive cell number in the vehicle group was significantly increased in comparison with the sham group, suggesting that astrogliosis was stimulated by ischemic injury. A remarkable decrease of GFAP-positive cells was counted in both

MSC-transplanted groups and the vehicle group (Fig. 7b). Furthermore, statistical analysis showed more reduction in the Sal-MSC group, which suggested that MSCs pretreated by salidroside inhibited neuroinflammation in the ischemic hippocampus.

Discussion

Stem cell therapy based on MSCs has emerged as a promising strategy for treating neurological diseases (Goldman 2016). However, previous studies have shown that the therapeutic effect of MSCs is limited due to their poor survival after transplantation (Li et al. 2016; Toma et al. 2002). Thus, improving the survival rate of MSCs is crucial for achieving a successful MSC-based therapy for cerebral ischemic injury. In the present study, we first found that salidroside had a significant effect on MSC proliferation, migration, and survival and reversed the injury induced by hypoxia–ischemia in vitro. Then, MSC pretreatment with salidroside revealed the reduced infarct rate and ameliorated pathological changes after transplantation. Furthermore, the results also demonstrated the elevated number of NeuN-positive cells and decreased number of GFAP-positive cells

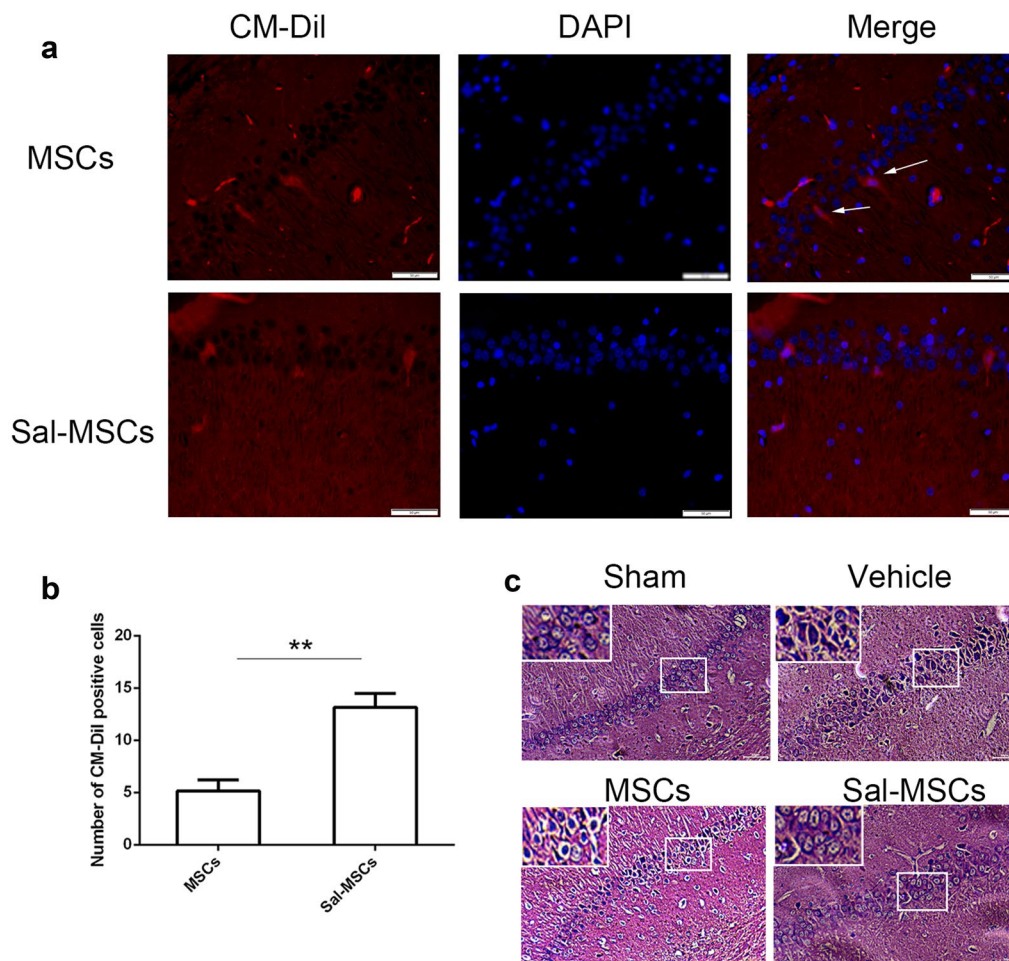


Fig. 5 Salidroside-pretreated MSCs alleviated pathological changes in the hippocampal CA1 area after ischemia. Transplanted MSCs labeled with CM-Dil (red) were observed in the hippocampal CA1

area (a). Quantification of CM-Dil positive cells. Columns represent mean \pm SD (n=3) (b). Morphological structures of the hippocampus were evaluated by HE staining (c). ** $P < 0.01$ versus MSCs group

in the ischemic hippocampus. Taken together, our findings clearly indicated that salidroside pretreatment has neuroprotective effects, which prompted further investigation of the underlying mechanisms.

Hypoxia–ischemia-induced neuroinflammation typically causes neurological damage and can occur during stroke, neonatal hypoxic-ischemic encephalopathy, and other diseases (Huang and Zhang 2019). CoCl_2 is a chemical compound, which is widely acknowledged as a classical stimulator to hypoxic-ischemic conditions. Here, we used serum-free medium with 100 $\mu\text{mol/l}$ CoCl_2 for hypoxia–ischemia induction. The proliferation, migration, and survival rate of MSCs were suppressed compared to normal conditions. Qin et al. (2008) reported that *Rhodiola rosea* could promote the proliferation and differentiation of neural stem cells in the hippocampus of the depressive rats and play a role in saving injured neurons of the hippocampus. Salidroside is a main component of *Rhodiola rosea*, which has been widely used as medicine in Asia and

Europe and possesses various medicinal properties, such as anti-inflammatory (Díaz Lanza et al. 2001), antioxidative (Ju et al. 2017), and neuroprotective effects (Chen et al. 2016; Gao et al. 2015; Palmeri et al. 2016). Studies have shown that salidroside can inhibit the apoptosis of many normal cells (Tan et al. 2009; Zhang et al. 2011). In addition, salidroside could alleviate hypoxia-induced viability impairment and apoptosis in neural stem cells and pulmonary arterial smooth muscle cells (Gui et al. 2017; Yan et al. 2018). Our results prompt the possibility that salidroside might be able to promote the survival and migration potential of MSCs and inhibit its apoptosis.

Studies have suggested that MSCs could control inflammation by reconstructing the neural microenvironment (Laroni et al. 2013). MSCs can secrete a series of growth factors, cytokines, chemokines, and various enzymes, playing an important role in cell growth and migration (Li and Zhao 2014). A report from Yun et al. (2013) has demonstrated that MSCs significantly inhibited the release of inflammatory

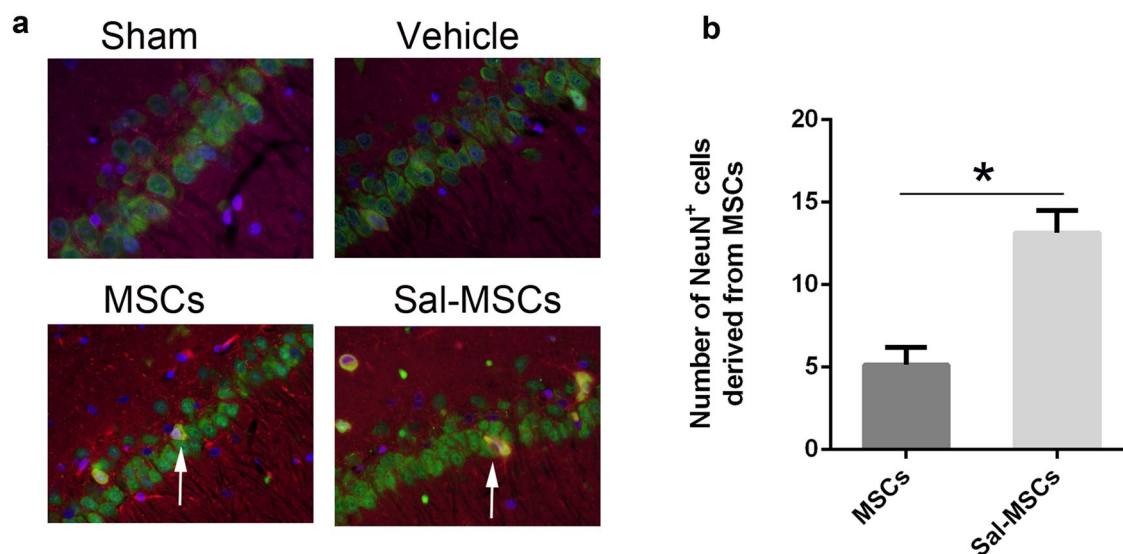


Fig. 6 Salidroside-pretreated MSCs increased the number of NeuN-positive cells in the ischemic hippocampus. CM-Dil positive cells (red) co-labeled with NeuN (green) were observed in the ischemic boundary zone with yellow fluorescence (a). Nuclei were counter-

stained with DAPI (blue). Quantitative analysis of CM-Dil co-labeled with NeuN-positive cells. Columns represent mean \pm SD (n=3) (b). * $P < 0.05$

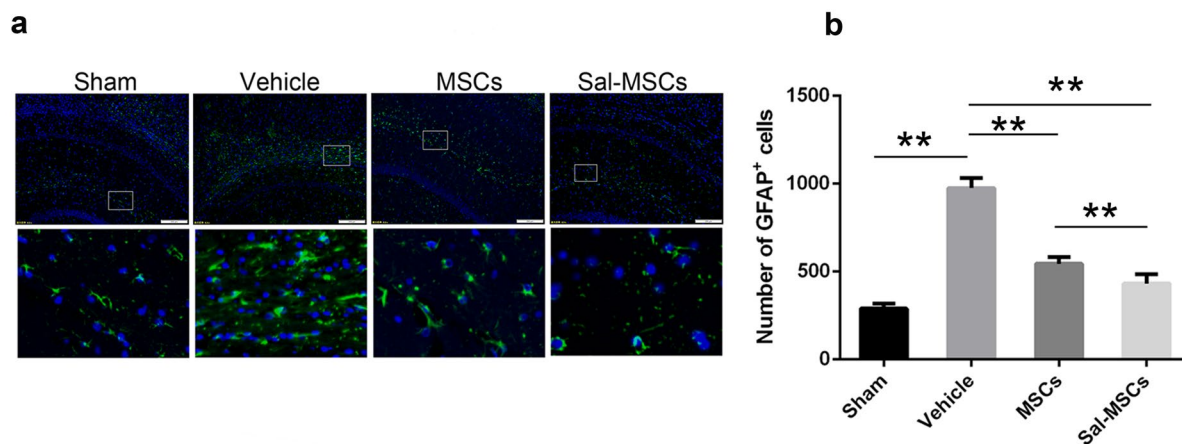


Fig. 7 Salidroside-pretreated MSCs decreased the number of GFAP-positive cells in the ischemic hippocampus. GFAP-positive cells in the ischemic boundary zone with immunolabeled green fluorescence. Nuclei were counterstained with DAPI (blue) (a). Quantitative analysis of GFAP-positive cells. Columns represent mean \pm SD (n=3) (b). ** $P < 0.01$

Nuclei were counterstained with DAPI (blue) (a). Quantitative analysis of GFAP-positive cells. Columns represent mean \pm SD (n=3) (b). ** $P < 0.01$

cytokines, alleviated cognitive impairment, and increased the survival rate of neurons and nerve regeneration. Furthermore, Ha et al. (2020) confirmed that salidroside could promote the survival of MSCs and combined to repair damaged neurons within spinal cord tissue. Our results showed that Sal-MSCs contributed to a significant decrease in the infarct rate and greatly enhanced neurogenesis in the focal area of MCAO rats. Several studies have revealed that salidroside could reduce cerebral ischemic injury (Han 2013; Shi et al. 2012; Zuo et al. 2018). According to previous studies, the

underlying mechanisms of salidroside against injury are due to inhibition of apoptosis and suppression of inflammation with good efficiency and low toxicity (Zhong et al. 2018). More importantly, the combination with MSCs may enhance the efficacy of salidroside. As shown in our in vivo results, the transplantation of Sal-MSCs strengthened the therapeutic efficiency by enhancing neurogenesis and inhibiting neuroinflammation in the hippocampus. Our results showed an increased number of NeuN-positive cells and a decreased number of GFAP-positive cells in the Sal-MSC group, which

demonstrated that the transplantation of Sal-MSCs could promote the differentiation of neurons in the lesions and alleviate inflammation caused by brain injury. Although further investigation is needed for confirmation before clinical application, our results showed that MSCs possessed curative effect to some extent, while salidroside-treated MSCs exert even better efficacy.

Conclusion

Our results clearly demonstrated that salidroside could promote the proliferation, migration, and reduced apoptosis of MSCs under hypoxic-ischemic conditions. Further in vivo experiments revealed that transplantation of Sal-MSCs strengthened the therapeutic efficiency by enhancing neurogenesis and inhibiting neuroinflammation in the hippocampal CA1 area after ischemia. These findings will open up the possibility of using this combination therapy for other MSC-based treatments.

Acknowledgements Not applicable.

Author contributions QY conceived the idea and designed the experiments. LPZ and PPY performed the experiments and wrote the manuscript. LXJ performed the animal experiments. ZYW and XHM performed the cellular experiment. GXW and JTY participated in part of the animal experiments. BJZ collected and analyzed the data. All authors read and approved the final manuscript.

Funding This work was financially supported by the National Natural Science Foundation of China (31570994); Project of Zhejiang Education Department (Y20163679).

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

References

Bai H, Wang CB, Ma XH, Wei YP, Xi R, Zhao Q, Zhang Q (2014) Effects of salidroside on proliferation of bone marrow mesenchymal stem cells. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 22:1072–1077. <https://doi.org/10.7534/j.issn.1009-2137.2014.04.035>

- Chen T et al (2016) Suppressing receptor-interacting protein 140: a new sight for salidroside to treat cerebral ischemia. *Mol Neurobiol* 53:6240–6250. <https://doi.org/10.1007/s12035-015-9521-7>
- Chen JX, Liu J, Hu F, Bi Y, Li M, Zhao L (2019) Genetic variants on chromosome 9p21 confer risks of cerebral infarction in the Chinese population: a meta-analysis. *Int J Immunopathol Pharmacol* 33:2058738419847852. <https://doi.org/10.1177/2058738419847852>
- Cunningham CJ, Redondo-Castro E, Allan SM (2018) The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. *J Cereb Blood Flow Metab* 38:1276–1292. <https://doi.org/10.1177/0271678x18776802>
- Díaz Lanza AM, Abad Martínez MJ, Fernández Matellano L, Recuero Carretero C, Villaescusa Castillo L, Silván Sen AM, Bermejo Benito P (2001) Lignan and phenylpropanoid glycosides from *Phillyrea latifolia* and their in vitro anti-inflammatory activity. *Planta Med* 67:219–223. <https://doi.org/10.1055/s-2001-12004>
- Feng G et al (2016) IGF-1 C domain-modified hydrogel enhances cell therapy for AKI. *J Am Soc Nephrol* 27:2357–2369. <https://doi.org/10.1681/asn.2015050578>
- Gao J et al (2015) Salidroside ameliorates cognitive impairment in a d-galactose-induced rat model of Alzheimer's disease. *Behav Brain Res* 293:27–33. <https://doi.org/10.1016/j.bbr.2015.06.045>
- Ge T et al (2016) Characterization of bone marrow-derived mesenchymal stem cells from dimethylallyl glycine-preconditioned mice: evaluation of the feasibility of dimethylallyl glycine as a mobilization agent. *Mol Med Rep* 13:3498–3506. <https://doi.org/10.3892/mmr.2016.4945>
- Goldman SA (2016) Stem and progenitor cell-based therapy of the central nervous system: hopes, hype, and wishful thinking. *Cell Stem Cell* 18:174–188. <https://doi.org/10.1016/j.stem.2016.01.012>
- Gui D et al (2017) Salidroside attenuates hypoxia-induced pulmonary arterial smooth muscle cell proliferation and apoptosis resistance by upregulating autophagy through the AMPK-mTOR-ULK1 pathway. *BMC Pulm Med* 17:191. <https://doi.org/10.1186/s12890-017-0477-4>
- Ha XQ, Yang B, Hou HJ, Cai XL, Xiong WY, Wei XP (2020) Protective effect of rhodiolside and bone marrow mesenchymal stem cells infected with HIF-1-expressing adenovirus on acute spinal cord injury. *Neural Regen Res* 15:690–696. <https://doi.org/10.4103/1673-5374.266920>
- Han T (2013) Effects of salidroside pretreatment on expression of tumor necrosis factor-alpha and permeability of blood brain barrier in rat model of focal cerebral ischemia-reperfusion injury. *Asian Pac J Trop Med* 6:156–158. [https://doi.org/10.1016/s1995-7645\(13\)60014-0](https://doi.org/10.1016/s1995-7645(13)60014-0)
- Hedhli J et al (2017) Multimodal assessment of mesenchymal stem cell therapy for diabetic vascular complications. *Theranostics* 7:3876–3888. <https://doi.org/10.7150/thno.19547>
- Huang L, Zhang L (2019) Neural stem cell therapies and hypoxic-ischemic brain injury. *Prog Neurobiol* 173:1–17. <https://doi.org/10.1016/j.pneurobio.2018.05.004>
- Jiang J, Dai J, Cui H (2018) Vitexin reverses the autophagy dysfunction to attenuate MCAO-induced cerebral ischemic stroke via mTOR/ULK1 pathway. *Biomed Pharmacother* 99:583–590. <https://doi.org/10.1016/j.biopha.2018.01.067>
- Ju L et al (2017) Salidroside, a natural antioxidant, improves β -cell survival and function via activating AMPK pathway. *Front Pharmacol* 8:749. <https://doi.org/10.3389/fphar.2017.00749>
- Laroni A, Novi G, Kerlero de Rosbo N, Uccelli A (2013) Towards clinical application of mesenchymal stem cells for treatment of neurological diseases of the central nervous system. *J Neuroimmune Pharmacol* 8:1062–1076. <https://doi.org/10.1007/s11481-013-9456-6>

- Li F, Zhao SZ (2014) Mesenchymal stem cells: potential role in corneal wound repair and transplantation. *World J Stem Cells* 6:296–304. <https://doi.org/10.4252/wjsc.v6.i3.296>
- Li Q, Guo Y, Chen F, Liu J, Jin P (2016) Stromal cell-derived factor-1 promotes human adipose tissue-derived stem cell survival and chronic wound healing. *Exp Ther Med* 12:45–50. <https://doi.org/10.3892/etm.2016.3309>
- Mangin G, Poittevin M, Charriaut-Marlangue C, Giannesini C, Merkoulova-Rainon T, Kubis N (2019) Glatiramer acetate reduces infarct volume in diabetic mice with cerebral ischemia and prevents long-term memory loss. *Brain Behav Immun* 80:315–327. <https://doi.org/10.1016/j.bbi.2019.04.009>
- Palmeri A, Mammana L, Tropea MR, Gulisano W, Puzzo D (2016) Salidroside, a bioactive compound of *rhodiola rosea*, ameliorates memory and emotional behavior in adult mice. *JAD* 52:65–75. <https://doi.org/10.3233/jad-151159>
- Pin-Barre C et al (2014) Acute neuromuscular adaptation at the spinal level following middle cerebral artery occlusion-reperfusion in the rat. *PLoS ONE* 9:e89953. <https://doi.org/10.1371/journal.pone.0089953>
- Qin YJ, Zeng YS, Zhou CC, Li Y, Zhong ZQ (2008) Effects of *Rhodiola rosea* on level of 5-hydroxytryptamine, cell proliferation and differentiation, and number of neuron in cerebral hippocampus of rats with depression induced by chronic mild stress. *Zhongguo Zhong Yao Za Zhi = China J Chinese Materia Medica* 33:2842–2846
- Shi TY et al (2012) Neuroprotective effects of salidroside and its analogue tyrosol galactoside against focal cerebral ischemia in vivo and H₂O₂-induced neurotoxicity in vitro. *Neurotox Res* 21:358–367. <https://doi.org/10.1007/s12640-011-9290-7>
- Tan CB, Gao M, Xu WR, Yang XY, Zhu XM, Du GH (2009) Protective effects of salidroside on endothelial cell apoptosis induced by cobalt chloride. *Biol Pharm Bull* 32:1359–1363. <https://doi.org/10.1248/bpb.32.1359>
- Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD (2002) Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105:93–98. <https://doi.org/10.1161/hc0102.101442>
- Yan R, Xu H, Fu X (2018) Salidroside protects hypoxia-induced injury by up-regulation of miR-210 in rat neural stem cells. *Biomed Pharmacother* 103:1490–1497. <https://doi.org/10.1016/j.biopha.2018.04.184>
- Yun HM et al (2013) Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1-42-infused mouse model of Alzheimer's disease. *Cell Death Dis* 4:e958. <https://doi.org/10.1038/cddis.2013.490>
- Zhang M et al (2012) Effect of salidroside on rat bone marrow mesenchymal stem cells differentiation into cholinergic nerve cells. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi = Chin J Reparative Reconstr Surg* 26:158–165
- Zhang K et al (2018) Enhanced therapeutic effects of mesenchymal stem cell-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl Mater Interfaces* 10:30081–30091. <https://doi.org/10.1021/acsami.8b08449>
- Zhang X et al (2019) Salidroside reduces inflammation and brain injury after permanent middle cerebral artery occlusion in rats by regulating PI3K/PKB/Nrf2/NFκB signaling rather than complement C3 activity. *Inflammation* 42:1830–1842. <https://doi.org/10.1007/s10753-019-01045-7>
- Zhang S, Chen X, Yang Y, Zhou X, Liu J, Ding F (2011) Neuroprotection against cobalt chloride-induced cell apoptosis of primary cultured cortical neurons by salidroside. *Mol Cell Biochem* 354:161–170. <https://doi.org/10.1007/s11010-011-0815-4>
- Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, Xiong Y (2015) Effect of exosomes derived from multipotential mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. *J Neurosurg* 122:856–867. <https://doi.org/10.3171/2014.11.jns14770>
- Zhao HB et al (2014a) Salidroside induces rat mesenchymal stem cells to differentiate into dopaminergic neurons. *Cell Biol Int* 38:462–471. <https://doi.org/10.1002/cbin.10217>
- Zhao HB et al (2014b) Salidroside induces neuronal differentiation of mouse mesenchymal stem cells through Notch and BMP signaling pathways. *Food Chem Toxicol* 71:60–67. <https://doi.org/10.1016/j.fct.2014.05.031>
- Zhong Z, Han J, Zhang J, Xiao Q, Hu J, Chen L (2018) Pharmacological activities, mechanisms of action, and safety of salidroside in the central nervous system. *Drug Des Dev Ther* 12:1479–1489. <https://doi.org/10.2147/ddt.s160776>
- Zomer HD, Vidane AS, Gonçalves NN, Ambrósio CE (2015) Mesenchymal and induced pluripotent stem cells: general insights and clinical perspectives. *Stem Cells and Cloning: Adv Appl* 8:125–134. <https://doi.org/10.2147/sccaa.s88036>
- Zuo W, Yan F, Zhang B, Hu X, Mei D (2018) Salidroside improves brain ischemic injury by activating PI3K/Akt pathway and reduces complications induced by delayed tPA treatment. *Eur J Pharmacol* 830:128–138. <https://doi.org/10.1016/j.ejphar.2018.04.001>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.