RESEARCH ARTICLE

HIF-1α promotes NSCs migration by modulating Slit2-Robo1 signaling after cerebral ischemia

Hua Ye[1], Min-rui Chen[2], Wan-fu Wu[3]

[1]*School of Public Health and Management, Research Center for Medicine and Social Development, Innovation Center for Social Risk Governance in Health, Chongqing Medical University, Chongqing 400016, China*
[2]*Department of Neurosurgery, the first people's hospital of Jiangbei district, Chongqing 400020, China*
[3]*Department of Neurology, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*

Correspondence: Hua Ye
E-mail: yehua121@hotmail.com
Received: April 14, 2017
Published online: June 26, 2017

Our previous studies have shown that transplantation of Hypoxia-inducible factor-1α (HIF-1α) gene modified neural stem cells (NSCs) reduced brain injury by improving the survival of NSCs and protecting the vascular system. HIF-1α plays pivotal roles during hypoxia, and its downstream pathways might be the primary mechanisms for the growth of NSCs. However, there are very few studies reported whether HIF-1α regulates NSCs migration. In this study, to test the hypothesis that HIF-1α modulates migration of NSCs after cerebral ischemia, we compared the injection of HIF-1α gene recombinant adenovirus, and control adenovirus in ischemia penumbra at 24 h after transient middle cerebral artery occlusion (tMCAO). BrdU labeled NSCs were transplanted in the lateral ventricle at the same time in both groups. The modified neurological severity score (NSS) was used to evaluate neurological deficits. Immunohistochemistry for HIF-1α, BrdU, Slit2 and Robo1 were performed. Comparing with vehicle group HIF-1α group showed better behavioral recovery on day 21 and 28. Expression of HIF-1α in HIF-1α group is higher than that in vehicle group. In HIF-1α group, more BrdU-positive cells were found than that in vehicle group. There are increased Slit2 in HIF-1α group. However, robo1, a receptor of Slits is decreased than that in vehicle group. Thus, we concluded that in cerebral ischemia rat model HIF-1α increased NSCs migration by inhibiting Slit2-Robo1 pathway, and improved the neurological behavior. In conclusion, our results indicate that HIF-1α may be a potential therapeutic target for ischemic stroke through promoting neuroregeneration.

Keywords: hypoxia-inducible factor-1α; neural stem cells; focal ischemia; migration; Slit2; Robo1

To cite this article: Hua Ye, *et al*. HIF-1α promotes NSCs migration by modulating Slit2-Robo1 signaling after cerebral ischemia. Receptor Clin Invest 2017; 4: e1549. doi: 10.14800/rci.1549.

Introduction

Neural stem cells (NSCs) are self-renewing multipotent progenitor cells that can generate three major neural lineages: neurons, astrocytes, and oligodendrocytes [1-3]. Endogenous NSCs can proliferate and migrate from the subventricular zone (SVZ) to the damaged area and differentiate to be neural lineages to play major roles in neurological recovery after ischemic stroke [4]. Thus, some NSCs lines are being used in clinical trials to treat cerebral infarction (CI) [5]. But,

post-engraftment NSCs survival is extremely poor, especially in the ischemic core of large infarctions [6]. Endogenous neurogenesis activating after infarction is insufficient to repair the damaged area of brain by itself [7]. Thus, promoting the proliferation and migration of endogenous/transplanted NSCs is a promising therapeutic target for ischemic infarction [8-10].

HIF-1α is constitutively expressed but is degraded rapidly by 26s proteasomes in normoxia in most cells. It is stabilized and transferred to the nucleus during hypoxia. HIF-1α regulates lots of genes involved in systemic, tissue and cellular adaption under low oxygen conditions [11]. It has been found that HIF-1α regulates hundreds of genes involved in adaption to low oxygen conditions including erythropoietin (EPO), vascular endothelial growth factor (VEGF), and several glycolytic enzymes, etc., which play crucial roles for neuroprotection and neurogenesis in the central nervous system [12, 13].

Studies have shown that HIF-1α expresses constitutively in neural stem/progenitor cells (NSPCs) in the adult SVZ and subgranular zone (SGZ) [14, 15]. Further study demonstrated that HIF-1α is implicated in stem cell maintenance by regulating Notch and Wnt/β-catenin differentiation pathways [16, 17]. However, few studies reported that if HIF-1α regulates NSCs migration. Our previous studies found that more NSCs migrated to per-ischemic area by transplanting HIF-1α gene modified NSCs [18]. However, further studies are needed to investigate the mechanism whether it is through increasing cell survival or/and by promoting cell migration.

In this study, we investigated whether HIF-1α can promote transplanted NSCs migration in transient middle cerebral artery occlusion (tMCAO) model in rats and the underline mechanisms.

Materials and Methods

The guidelines for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, China (1985) was followed in this study.

Focal cerebral ischemia rat model

Male Sprague-Dawley rats weighting from 230 g to 250 g were used. Before surgery rats were housed individually and maintained at a room temperature of 25 °C and had free access to food and water under the condition of a 12-hour light-dark cycle. Rats were initially anesthetized with 50 mg/kg of ketamine and 10 mg/kg of xylazine intraperitoneally. In this study tMCAO model was induced. Briefly, the right common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were isolated. A 3 cm length of 4-0 nylon suture with a slightly enlarged and rounded tip was advanced from the ECA into the lumen of the ICA until it blocked the origin of the middle cerebral artery (MCA). The distance from the tip of the suture to the bifurcation of the common carotid artery is 18.5 to 19.5 mm. Reperfusion was performed by withdraw the suture until the tip cleared the lumen of the ECA after 90 min.

Cell cultures

The hippocampus of Embryonic Spargue-Dawley rats (E14) was dissected. The tissues were transferred to cold PBS, minced with scissors, and dissociated with Pasteur pipette. Then the suspension was centrifuged at 75 × *g* in a 10-ml centrifuge tube for 10 minutes. At last cells were seeded (final density: 1×10^5 viable cells/ml) in Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1; Gibco) plus B27 supplement (2 ml/100 ml, Gibco), bFGF (20 ng/ml, Sigma) and EGF (20 ng/ml, Sigma) and cultured at a humidified atmosphere of 5% CO_2/95% air for 3 to 7 days. After passaging four generations, neurospheres were dissociated into single cells by incubation in 0.1% trypsin-ethylenediamine tetra-acetic acid at 37°C for 2 minutes and then centrifugation in 10 ml of DMEM/F12 medium containing 4% bovine serum albumin at 110 × *g* for 5 minutes. Neurospheres from single cells were transferred to a poly-l-ornithine-coated 96-well dish and cultured in the same medium.

Transplantation procedures

Recombinant adenovirus carrying HIF-1α gene and green florescent protein (GFP) gene (Ad-HIF-1α) have been prepared using AdEasy system. The titer of Ad-HIF-1α was between 10^9 and 10^{10} PFU/ml. On day 1 after MCAO, animals with NSS scores of 7 to 12 were randomly divided into two groups (n=12, respectively): NSCs+Vehicle (Vehicle group), NSCs+ Ad-HIF-1α (HIF-1α group). All NSCs had been labeled with 5 mmol/L BrdU for 3 days before transplantation. Rats were anesthetized with equithesin (3 ml/kg administered intraperitoneally) and transferred to a stereotaxic apparatus. Using aseptic technique a 2 to 5 mm incision was made in the scalp 1.5 mm lateral to the bregma. Using a dental drill a burr hole was made in the bone 3 mm lateral to the bregma and about 10 μl of NSCs (1×10^6 cells) were slowly injected during 10 min into the lateral ventricle at a depth of 3.5 mm from the surface of the brain for the two groups. The needle was retained in the brain for an additional 5 min before retraction.

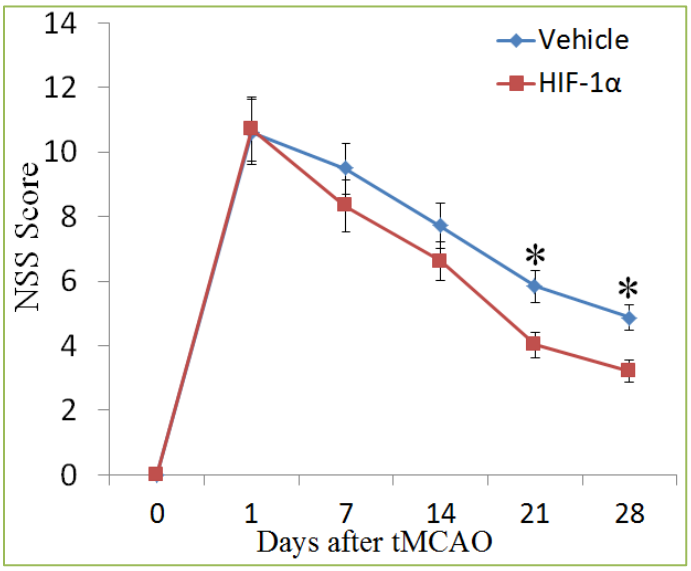

Figure 1. Behavioral tests. NSS score in HIF-1α group was superior to Vehicle group on post-ischemia day 21 and 28. There is no difference on day 7 and 14. (*: $p < 0.05$).

After that at a depth of 2.0 mm from the surface of the brain 10 μl Ad or 10 μl Ad-HIF-1α was injected to the ischemic area respectively.

Behavioral testing

The modified neurological severity score (NSS) [19] was used to evaluate neurological deficit of the 2 groups. The NSS is a composite of sensory (visual, tactile, proprioceptive), motor (muscle status, abnormal movement), reflex, and balance tests. The score was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18), with higher score, with more severe injury.

Histological analysis

On day 7, 14, 21 and day 28 after ischemia, rats were deeply anesthesia and transcardially perfused for histological analysis before euthanizing. After 24 h fixation in 4% paraformaldehyde, brains were processed for paraffin sections (5 μm). Paraffin sections were deparaffinized in xylene, rehydrated through graded alcohol, and processed for antigen retrieval by boiling in 10 mM citrate buffer (pH 6.0) for 12 to 15 min in PT module. Sections were incubated in 0.3% H2O2 in 50% methanol for 30 min at room temperature to quench endogenous peroxidase. To block nonspecific binding, sections were incubated in 3% BSA for 30 min, and then a biotin blocking system (Dako) was used to block endogenous biotin. Sections then were incubated with Anti-HIF-1α (1:200, Boster, China) to evaluate the expression of HIF-1α. To identify transplanted cells, Mouse Anti-BrdU (1:200, Boster) was used. The number of BrdU-positive cells was determined by direct counting of one tenth of whole sections. To investigate the expression of Slit2 and Robo1, Anti-Slit2 (1:200, Santa Cruz) and Anti-Robo1 (1:200, Santa Cruz) were performed.

Statistical analysis

Data were showed as means ± sd. Data were analyzed by repeated measures of analysis of variance (ANOVA), and unpaired Student's t-test, if they were normally distributed (Kolmogorov-Smirov test, $p>0.05$). A probability value of less than 5% was considered statistically significant.

Results

Ad-HIF-1α group shows better functional recovery

In this study, we compared NSS scores (n=12 for each group) to investigate if the transplantation of Ad-HIF-1α in infarction area and NSCs in lateral ventricle could get better neurological recovery. There were no significant differences between the two groups in NSS scores either before MCAO or on day 1 after MCAO. In both groups, NSS demonstrated a progressive recovery from week 1 to week 4 after MCAO. Ad-HIF1-α treatment showed a significant improvement in NSS scores compared with vehicle group on post ischemia day 21 (P21) and P28 (Fig. 1). No significant difference was found on P7 and P14 (Fig. 1). During the 4 weeks body weights of the two groups had no significant difference.

Ad-HIF-1α treatment increases HIF-1α expression

HIF-1α expression of HIF-1α group was increased compared with that in the vehicle group on day 7, d 14 and d 21 after tMCAO. HIF-1α Expression was sustained up to about 21 days (Fig. 2). HIF-1α positive staining was located in both cytoplasm (red arrow) and nuclei (black arrow). It was found in both the peri-ischemic area and core region after MCAO. Both neurons and glia cells expressed HIF-1 α (Fig. 2). HIF-1α -positive staining was not found in the non-ischemic hemisphere (data not shown). The number of HIF-1α positive cells in HIF-1α group was significantly increased compared to that in vehicle group (Fig. 2 G, $P<0.01$).

HIF-1α promotes NSCs migration to peri-ischemic area

Four weeks after ischemia, transplanted NSCs cells in lateral ventricle were detected by immunofluorescence for BrdU. BrdU-positive cells were found in the peri-ischemic area. The number of BrdU-positive cells was significantly

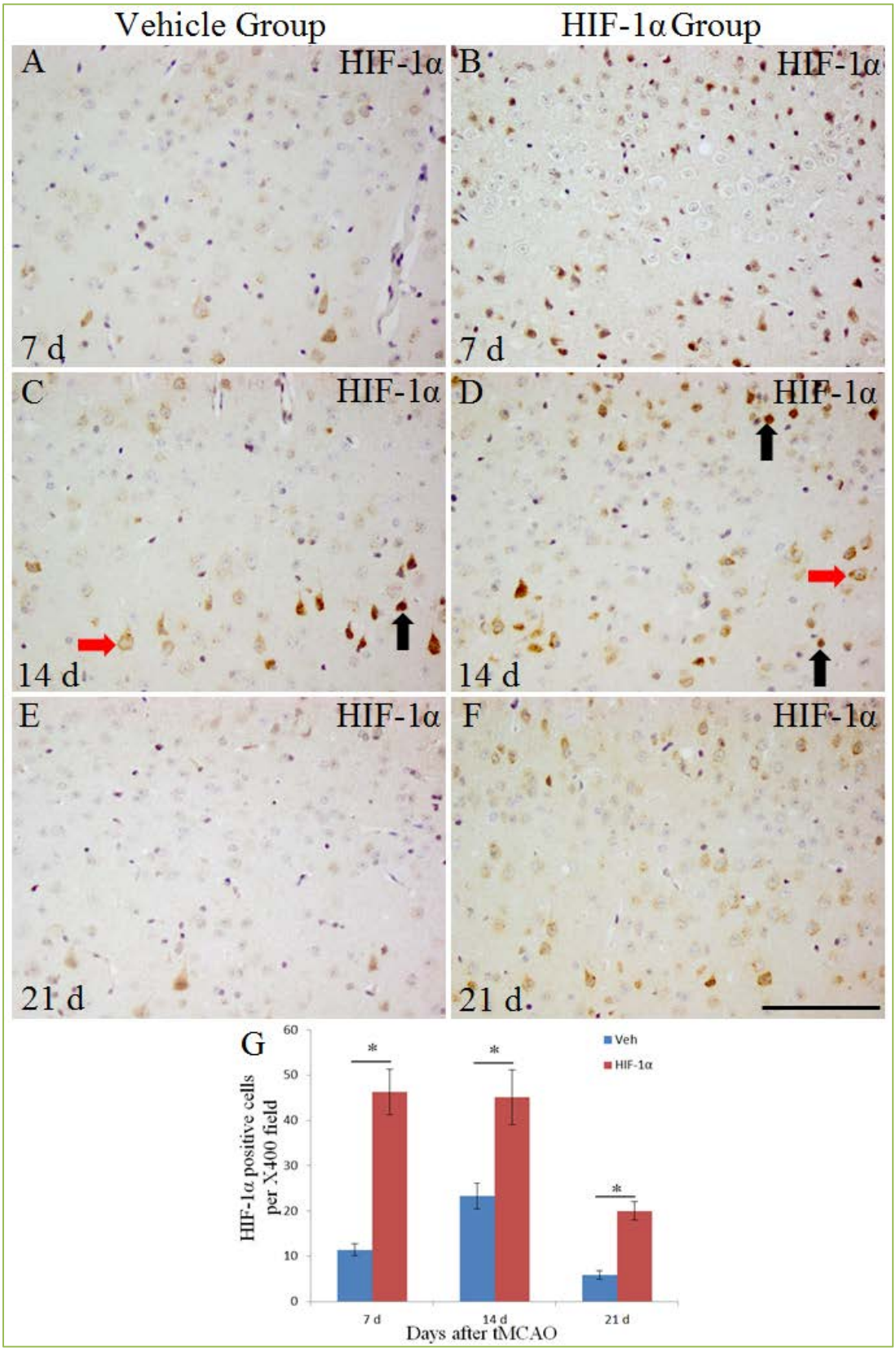

Figure 2. HIF-1α expression in peri-ischemic area on day 7, 14 and 21 after tMCAO. (A, C, E) Vehicle group; (B, D, F) HIF-1α group. Red arrows show cytoplasmic positive staining. Black arrows show nuclear positive staining. Bar=50 μm. (G) Quantitative analysis of HIF-1α positive cells in ischemic penumbra tissue. $p < 0.05$ compared with Vehicle group (*).

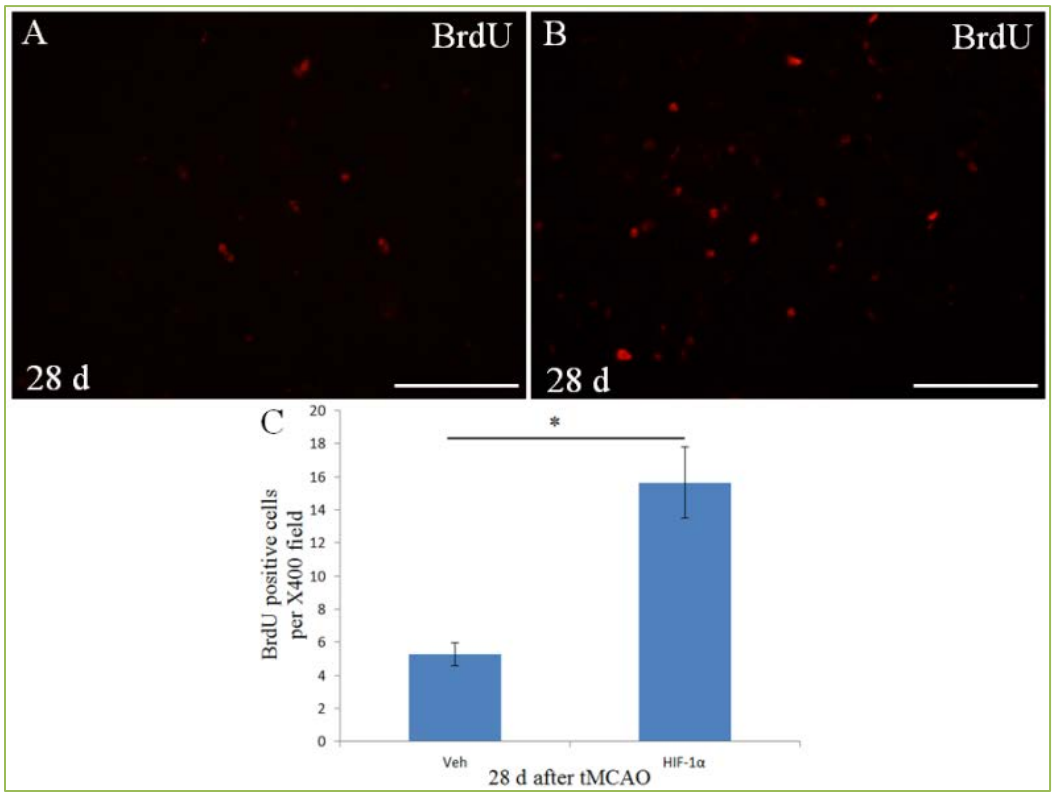

Figure 3. BrdU staining in peri-ischemic area on day 28 after tMCAO. (A) Vehicle group, (B) HIF-1α group. Bar=50μm. (C) Quantitative analysis of BrdU positive cells in ischemic penumbra tissue. Bars represent mean ± SD. Compared with Vehicle group (*: $p < 0.05$).

higher in HIF-1α group than that in vehicle group (Fig. 3).

HIF-1α enhances expression of Slit2 in peri-ischemic area

It is reported that astrocytes in peri-ischemic area express Slit2. Our study showed that Slit2-positive cells are astrocyte-like cells. In vehicle group it was detected on day 7, day 14 and day 21 after tMCAO. On day 14, the expression level is the highest (Fig 4, A, C and E). In the HIF-1α group Slit2 was significant increased compared with that in the vehicle group on day 7, day 14 and day 21 after tMCAO (Fig 4, B, D, F and G).

HIF-1α inhibits expression of Robo1 after tMACO

To determine if HIF-1α regulated the expression of Robo1 in peri-ischemic area, we calculated the number of Robo1-positive cells. After tMCAO, Robo1 expression was sustained up to about 21 days. On day 21, Robo1 expression was higher than that on day 7 and day 14 after ischemia (Fig 5, A, C and E). Robo1 expression in the HIF-1α group was decreased compared with that in the vehicle group on day 7, d 14 and d 21 after MCAO(Fig 5, B, D and F). The number of Robo1 positive cells in HIF-1α group was significantly decreased compared to that in vehicle group (Fig 5, G. $P<0.01$).

Discussions

This study has tested the hypothesis that HIF-1α regulates migration of NSCs through modulating Slit2-Robo1 signaling after cerebral ischemia. To evaluate NSCs migration, tMCAO rats were treated with recombinant adenovirus with HIF-1α gene and NSCs were transplanted into lateral ventricle. The data demonstrated that HIF-1α increased migration of transplanted NSCs leading to a better functional recovery. Combine HIF-1α and NSCs treatment showed more benefits than NSCs treatment. HIF-1α upregulated expression of Slit2 in peri-ischemic area, meanwhile, it downregulated expression of Robo1 (a receptor of Slit2). These results suggest that HIF-1α could promote migration of NSCs after cerebral ischemia by inhibiting Slit2-Robo1 pathway. HIF-1α may be a potential therapeutic agent for cerebral ischemia by promoting NSCs migration.

To evaluate the change of HIF-1α expression after treatment, HIF-1α immunohistochemistry was performed in this study. The result showed that HIF-1α expression in the HIF-1α group was all increased compared with that in the

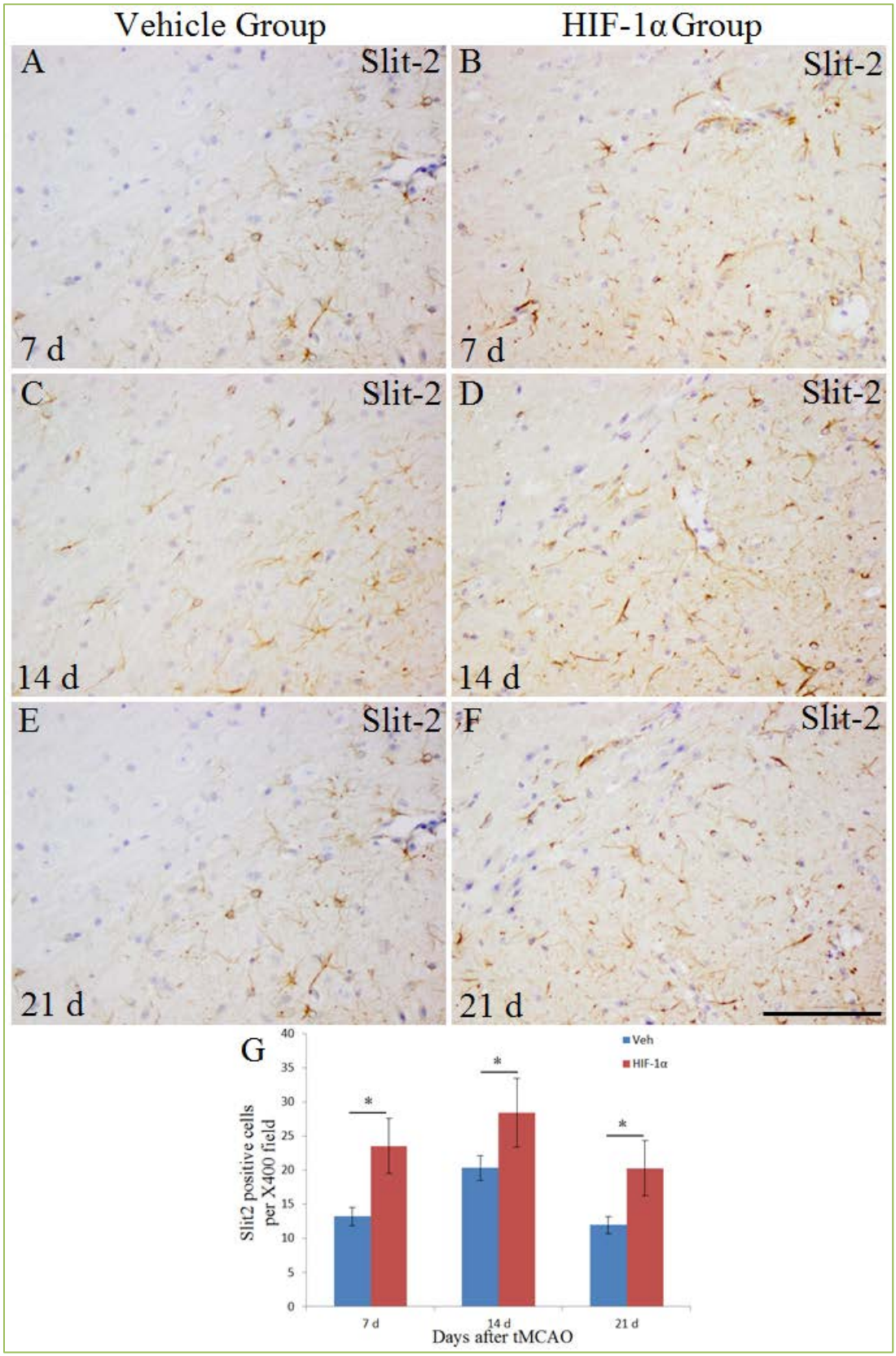

Figure 4. Immunohistochemical detection of Slit2 in ischemic penumbra on day 7, 14 and 21. (A, C, E) Vehicle group; (B, D, F) HIF-1α group. Slit2 expresses mostly on astrocytes. Bar=50 μm. (G) Quantitative analysis of Slit2 positive cells. $p < 0.05$ compared with Vehicle group (*).

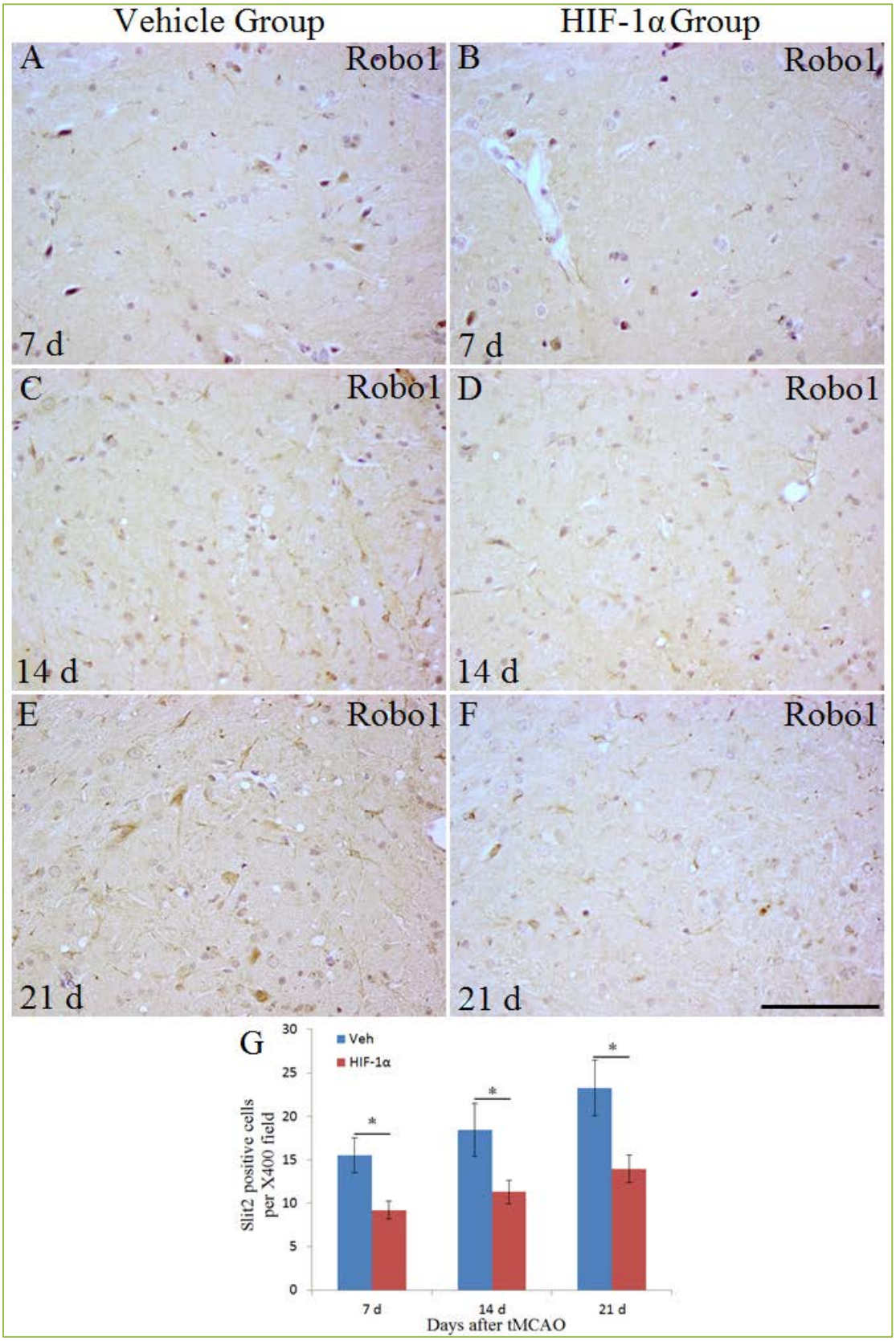

Figure 5. expression of Robo1 in peri-ischemic area on day 7, 14 and 21 after tMCAO. (A, C, E) Vehicle group; (B, D, F) HIF-1α group. Bar=50 μm. (G) Quantitative analysis of HIF-1α positive cells in ischemic penumbra tissue. (*: $p < 0.05$).

vehicle group on day 7, d 14 and d 21 after MCAO. HIF-1α, a key regulator in hypoxia, determines the fate of cells during cerebral ischemia. After hydroxylation and ubiquitination, HIF-1α is quickly degraded via the ubiquitin-dependent proteasomal (26S) degradation pathway under normoxic conditions. However, under hypoxic conditions, it is accumulated due to hydroxylation inhibition [20, 21]. HIF-1α has a lot of target genes, including VEGF, EPO, several glycolytic enzymes and stromal-derived growth factor1. These target genes have effects on neuroprotection, neurogenesis and angiogenesis in the brain [22, 23]. Neuron-specific HIF-1α deficient mice showed increased brain damage following MCAO [24]. HIF-1α conditional knock-out midbrain-derived neural precursor cells showed midbrain-specific impairment of survival and proliferation [25]. In this study, HIF-1α expression was increased by treatment at different time point. Upregulated HIF-1α may contribute to better neurologic function recover by modulating its downstream pathways.

To evaluate the migration of transplanted NSCs, BrdU was used to label NSCs before transplantation. Immunofluorescence for BrdU was performed in order to detect transplanted NSCs. The results demonstrated that more transplanted NSCs migrated to peri-ischemic area after HIF-1α treatment. Cerebral ischemia causes a series of reactions resulting in the death of neurons and then nerve function damage. NSCs are undifferentiated cells sourced from ectodermal tissue. NSCs therapy has emerged as a novel and promising candidate for the treatment of stroke due to their capacity to self-renewal and to differentiate into a range of tissues [26]. Endogenous NSCs activated after injury are insufficient to repair the damaged area of brain by themselves in animal models [7]. Neurogenesis enhanced by exogenous factor regulating the proliferation and migration of endogenous NSCs have been related with improved functional recovery in many studies [27, 28]. It represents a promising therapeutic approach for treating stroke. This study gave evidence that HIF-1α promoted the migration of NSCs transplanted in lateral ventricle to per-ischemic area.

To investigate how HIF-1α promoted the migration of NSCs, Slit2 and its receptor, Robo1 were checked. The results showed that HIF-1α upregulated expression of slit2 and downregulated Robo1 in peri-ischemic area. Slit, identified in Drosophila embryo, is a gene involved in the patterning of larval cuticle. There are three slit genes (slit1, slit2 and slit3) in mammals. Slits are ligands of its receptors, Robol-Robo3. An important function for Slits and Robos is the control of cell migration in the nervous system (both neurons and glia) and in several other tissues. As for axons, Slits were found to be important regulators of the behavior of migrating cells at the midline. Migrating cells can either be attracted or repelled by Slits. When Slit2 binds to Robo1, it repels cell migration [29]. Some studies have already reported that the Slit2/Robo1 signaling inhibits cell migration [30]. This study demonstrated that HIF-1α increased expression of Slit2; on the other hand it decreased Robo1 expression. So there were less Robo1 binding to Slit2. As a result, there were more NSCs transplanted in lateral ventricle migrated to peri-ischemic area.

To summarize, our findings demonstrate that HIF-1α promoted NSCs migration by inhibiting Slit2-Robo1 pathway, and thus improved the neurological behavior in cerebral ischemia rat model. The results indicate that HIF-1α may be a potential therapeutic target for ischemic stroke through promoting neuroregeneration.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgements

This study was funded by Chongqing Municipal Health Bureau and Chongqing Education Committee(KJ120305).

References

1. Narayanan G, Yu YH, Tham M, Gan HT, Ramasamy S, Sankaran S, *et al*. Enumeration of Neural Stem Cells Using Clonal Assays. J Vis Exp. 2016. doi: 10.3791/54456.
2. De Feo D, Merlini A, Laterza C, Martino G. Neural stem cell transplantation in central nervous system disorders: from cell replacement to neuroprotection. Curr Opin Neurol 2012; 25:322-333.
3. Kokaia Z, Martino G, Schwartz M, Lindvall O. Cross-talk between neural stem cells and immune cells: the key to better brain repair? Nat Neurosci 2012; 15:1078-1087.
4. Lee WD, Wang KC, Tsai YF, Chou PC, Tsai LK, Chien CL. Subarachnoid Hemorrhage Promotes Proliferation, Differentiation, and Migration of Neural Stem Cells via BDNF Upregulation, PLoS One 2016; 11: e0165460. doi: 10.1371/journal.pone.0165460.
5. Koh SH, Lo EH. The Role of the PI3K Pathway in the Regeneration of the Damaged Brain by Neural Stem Cells after Cerebral Infarction, J Clin Neurol 2015; 11:297-304.
6. Andres RH, Choi R, Steinberg GK, Guzman R. Potential of adult neural stem cells in stroke therapy. Regen Med 2008; 3:893-905.
7. Göritz C, Frisén J. Neural stem cells and neurogenesis in the adult. Cell Stem Cell 2012; 10: 657-659.
8. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, *et al*. Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 2006;442:823-826.

9. Klein R, Blaschke S, Neumaier B, Endepols H, Graf R, Keuters M, *et al*. The synthetic NCAM mimetic peptide FGL mobilizes neural stem cells in vitro and in vivo. Stem Cell Rev 2014;10:539-547.
10. Hucklenbroich J, Klein R, Neumaier B, Graf R, Fink GR, Schroeter M, *et al*. Aromatic-turmerone induces neural stem cell proliferation in vitro and in vivo. Stem Cell Res Ther 2014; doi:10.1186/scrt500.
11. Weidemann A, Johnson RS. Biology of HIF-1alpha. Cell Death Differ 2008; 15: 621-627.
12. Greenberg DA, Jin K. From angiogenesis to neuropathology. Nature 2005; 438:954-959.
13. Speer R, Ratan RR. Hypoxic Adaptation in the Nervous System: Promise for Novel Therapeutics for Acute and Chronic Neurodegeneration. Adv Exp Med Biol 2016; 903:221-243.
14. Mazumdar J, O'Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS, *et al*. O2 regulates stem cells through Wnt/beta-catenin signalling. Nat Cel lBiol 2010;12:1007-1013.
15. Roitbak T, Surviladze Z, Cunningham LA. Continuous expression of HIF-1alpha in neural stem/progenitor cells. Cell Mol Neuro boil 2011;31:119-133.
16. Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, Zhao C, *et al*. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol 2005; 6:314-322.
17. Kaidi A, Williams AC, Paraskeva C .Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. Nat Cell Biol 2007; 9:210-217.
18. Wu W, Chen X, Hu C, Li J, Yu Z, Cai W. Transplantation of neural stem cells expressing hypoxia-inducible factor-1alpha (HIF-1alpha) improves behavioral recovery in a rat stroke model. J Clin Neurosci. 2010;17:92-95.
19. Bertram CM1, Hawes SM, Egli S, Peh SL, Dottori M, Kees UR, *et al*. Effective adenovirus-mediated gene transfer into neural stem cells derived from human embryonic stem cells. Stem Cells Dev 2010;19:569-578.
20. Kallio PJ, Wilson WJ, O'Brien S, Makino Y, Poellinger L. Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. J Biol Chem 1999; 274, 6519-6525.
21. Huang LE, Bunn HF. Hypoxia-inducible factor and its biomedical relevance. J Biol Chem 2003;278, 19575-19578.
22. Jang W, Kim HJ, Li H, Jo KD, Lee MK, Yang HO. The Neuroprotective Effect of Erythropoietin on Rotenone-Induced Neurotoxicity in SH-SY5Y Cells Through the Induction of Autophagy. Mol Neurobiol 2016; 53:3812-3821.
23. Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. Cell Mol Life Sci 2013;70:1753-1761.
24. Baranova O, Miranda LF, Pichiule P, Dragatsis I, Johnson RS, Chavez JC. Neuron-specific inactivation of the hypoxia inducible factor 1 increases brain injury in a mouse model of transient focal cerebral ischemia. J Neurosci 2007 27:6320- 6332.
25. Milosevic J, Maisel M, Wegner F, Leuchtenberger J, Wenger RH, Gerlach M, *et al*. Lack of hypoxia-inducible factor-1 alpha impairs midbrain neural precursor cells involving vascular endothelial growth factor signaling. J Neurosci 2007; 27:412-421.
26. Hao L, Zou Z, Tian H, Zhang Y, Zhou H, Liu L. Stem Cell-based therapies for ischemic stroke. Biomed Res Int 2014; 2014:468748. doi: 10.1155/2014/468748.
27. Hermann DM, Chopp M. Promoting brain remodeling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol 2012; 11, 369-380.
28. Lu H, Song X, Wang F, Wang G, Wu Y, Wang Q, *et al*. Hyperexpressed Netrin-1 Promoted Neural Stem Cells Migration in Mice after Focal Cerebral Ischemia. Front Cell Neurosci 2016; 10: 223. eCollection 2016. DOI:10.3389/fncel.2016.00223.
29. Hu H. Chemorepulsion of neuronal migration by slit2 in the developing mammalian forebrain. Neuron 1999; 23:703-711.
30. Mertsch S, Schmitz N, Jeibmann A, *et al*: Slit2 involvement in glioma cell migration is mediated by Robo1 receptor. J Neurooncol 2008; 87: 1-7.