ORIGINAL ARTICLE

The Expression of FOXJ1 in Neurogenesis after Transient Focal Cerebral Ischemia

Yabo Huang, Zheng Xu, Jie Cao, Haibo Cao, Shiming Zhang

ABSTRACT: ***Objective and Background:*** FOXJ1 is a member of the Forkhead/winged-helix (Fox) family of transcription factors, which is required for the differentiation of the cells acting as adult neural stem cells which participate in neurogenesis and give rise to neurons, astrocytes, oligodendrocytes. The expression pattern of FOXJ1 in the brain after cerebral ischemia has so far not been described. In the current study, we investigated the expression pattern of FOXJ1 in the rat brain after cerebral ischemia by animal model. ***Methods:*** We performed a middle cerebral artery occlusion (MCAO) model in adult rats and investigated the expression of FOXJ1 in the brain by Western blotting and immunochemistry; double immunofluorescence staining was used to analyze FOXJ1's co-expression with Ki67. ***Results:*** Western blot analysis showed that the expression of FOXJ1 was lower than normal and sham-operated brain after cerebral ischemia, but the level of FOXJ1 gradually increased from Day 1 to Day 14. Immuohistochemical staining suggested that the immunostaining of FOXJ1 deposited strongly in the ipsilateral and contralateral hemisphere in the cortical penumbra (CP). There was no FOXJ1 expression in the ischemic core (IC). The positive cells in the cortical penumbra might migrat to the ischemic core. In addition, double immunofluorescence staining revealed that FOXJ1 was co-expressed with MAP-2 and GFAP, and Ki67 had the co-localization with NeuN, GFAP, and FOXJ1. ***Conclusions:*** All our findings suggest that FOXJ1 plays an important role on neuronal production and neurogenesis in the adult brain after cerebral ischemia.

RÉSUMÉ: L'expression de FOXJ1 dans la neurogenèse après un accès ischémique focal transitoire. ***Objectif et contexte :*** FOXJ1 est un membre de la famille de facteurs de transcription Forkhead/winged-helix (Fox). Il est requis pour la différenciation des cellules agissant comme cellules souches nerveuses adultes qui participent à la neurogenèse et donnent naissance à des neurones, des astrocytes et des oligodendrocytes. L'expression de FOXJ1 dans le cerveau suite à un accès ischémique focal transitoire n'a pas été décrite à ce jour. Dans cette étude, nous avons exploré l'expression de FOXJ1 dans un modèle animal, le cerveau de rat, suite à une ischémie cérébrale. ***Méthode :*** Nous avons effectué une occlusion de l'artère cérébrale moyenne chez des rats adultes et nous avons examiné l'expression de FOXJ1 dans le cerveau par buvardage Western et immunohistochimie. Nous avons utilisé le double marquage par immunofluorescence pour analyser la coexpression de FOXJ1 et de Ki67. ***Résultats :*** L'analyse par buvardage Western a montré que l'expression de FOXJ1 était plus faible dans le cerveau de rat qui avait subi une ischémie cérébrale que dans le cerveau normal ou le cerveau qui avait subi une intervention factice. Cependant, le niveau de FOXJ1 augmentait graduellement du jour 1 au jour 14 après l'ischémie. La coloration immunohistochimique de FOXJ1 était intense dans la zone de pénombre corticale de l'hémisphère ipsi et contralatéral. Il n'y avait pas d'expression de FOXJ1 dans le centre de la zone ischémique. Il est possible que les cellules positives dans la zone de pénombre corticale puissent migrer vers le centre de la zone ischémique. De plus, une double coloration par immunofluorescence a montré une coexpression de FOXJ1 et MAP-2 et GFAP, et une colocalisation de Ki67 et de NeuN, GFAP et FOXJ1. ***Conclusions :*** Ces observations sont en faveur d'un rôle important de FOXJ1 dans la production neuronale et la neurogenèse dans le cerveau adulte à la suite d'une ischémie cérébrale.

Can J Neurol Sci. 2013; 40: 403-409

Stroke is the second most common cause of death after ischemic heart disease and a major cause of disability worldwide[1]. Ischemic stroke constitutes about 80% of all strokes. Ischemic stroke is a disorder involving multiple mechanisms of injury progression including activation of glutamate receptors, release of proinflammatory cytokines, nitric oxide, free oxygen radicals and proteases. Stroke causes brain injury and infarction of tissue with activation of an inflammatory response that includes activation of microglia and astrocytes[2-5] However, neurogenesis persists in the adult mammalian brain in mainly two restricted areas, the subventricular zone (SVZ) of the lateral ventricles and the detate gyrus of the hippocampus[6]. Studies of experimental stroke in adult rodents and primates demonstrated that ischemic insults promote neurogenesis in the forebrain subventricular and hippocampal dentate gyrus germinative zones, and induce endogenous neural stem cells / progenitors to migrate to areas of damage and form neurons in otherwise dormant forebrain regions of the mature brain[7].

FOXJ1 is a member of the forkhead/winged-helix (Fox) family of transcription factors, which has a conserved 100 amino acid DNA binding domain and plays important roles in cilia formation of the respiratory, reproductive, and central nervous

From the Department of Neurosurgery, the First Affiliated Hospital of Soochow University, Suzhou, China.

Received August 30, 2012. Final Revisions Submitted November 29, 2012.

Correspondence to: Shiming Zhang, Department of Neurosurgery, the First Affiliated Hospital of Soochow University, Suzhou, 215006, China.
Email: zhang.shiming@yahoo.cn.

systems[8-10]. FOXJ1 plays a direct role in the development of motile cilia[10-12], and motile cilia are important elements of ependymal cell differentiation and function in the central nervous system[13,14]. Previous experiment studies demonstrated that FOXJ1 might be required for the differentiation of the cells acting as adult neural stem cells which participate in neurogenesis and give rise to neurons, astrocytes, oligodendrocytes. These cells might migrate to the lesion region following stimulation of injury, to compensate for the loss of neuronal function caused by traumatic brain injury (TBI)[15]. Recent studies have revealed that FOXJ1-promoter active cells in the spinal canal and SVZ participate in neurogenesis and gliogenesis in the injured spinal cord and in response to stroke[16,17]. There is also evidence that FOXJ1 is required for postnatal differentiation of ependymal cells and a subset of astrocytes in the SVZ and the subpopulation of astrocytes has the ability of self-renewal and neurogenic potential to differentiate into astrocytes, oligodendrocytes and neurons[18]. In the absence of FOXJ1 expression, all ependymal cells within the lateral ventricles fail to differentiate during postnatal periods, regardless of their position. FOXJ1 may play a role in a confined population of neural progenitors in addition to its role in the generation of motile cilia in ependymal cells[19]. Most of these studies have provided reliable evidences that FOXJ1 plays an important role in neuronal production and neurogenesis which persist in the adult brain after ischemia injury. We can speculate that FOXJ1 might contribute to the pathophysiological progression after cerebral ischemia.

In the present study, we investigated the expression of FOXJ1 in the rat brain after transient focal cerebral ischemia, with the aim to understand the role of FOXJ1 in progress after cerebral ischemia.

Materials and Methods

Animals and Transient Focal Cerebral Ischemia

Adult male Sprague-Dawley rats weighing 250-280g were obtained from the Model Animal Research Center of Soochow University. Animal care and all procedures were carried out in accordance with the National Institutes of Health (NIH) Guide for the Use and Care of Laboratory Animals. The study was approved by the local ethical review committee.

Rats were fasted overnight but had access to water *ad libitum* before induction of cerebral ischemia. Yransient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) as previously described by Belayev et al[20] with a few modifications. Briefly, rats were anesthetized with 10% chloral hydrate (300-400mg/kg, intraperitoneally). Their rectal temperature was maintained between 36.5-C and 37.5-C using an Homeothermic Blanket Control Unit (Harvard Apparatus, Holliston, MA, USA). The intraluminal suture method was used to occlude the middle cerebral artery (MCA)[21,22]. The right common carotid artery was exposed through a ventral midline neck incision and was carefully dissected free from vagus nerve and fascia, from its bifurcation to the base of the skull. The superior thyroid, ascending pharyngeal, and distal branches of the external carotid artery (ECA) were then dissected, ligated, and divided to create a 5-mm ECA stump. The internal carotid artery was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was dissected and ligated close to its origin with a 6-0 nylon suture. Next, a 4-0 monofilament nylon suture with a rounded tip was inserted through an arteriotomy in the ECA stump into the internal carotid artery and advanced to the MCA origin to occlude it. A 6-0 silk suture was tied around the intraluminal nylon suture as it passed through the ECA stump to prevent bleeding. Occlusion of the MCA was ascertained by inserting the suture to a predetermined length of 19 to 20 mm from the carotid bifurcation and feeling for resistance as the rounded suture tip approached the proximal anterior cerebral artery, which has a relatively narrower caliber. The cervical wound was then closed in layers. Two hours after occlusion; the filament was withdrawn to allow reperfusion. In sham-operated rats, the thread did not reach the origin of the MCA and was removed two hours after occlussion. Animals with no immediate observable deficits after ischemia, those that died before 24 hours, and those with subarachnoid hemorrhage at the time of death were excluded from analysis. Experimental animals were killed at 1, 2, 3, 4, 5, 7 and 14 days after occlusion. Sham-operated rats were sacrificed as soon as the thread was removed.

Western Blot

After administratior of an overdose of chloral hydrate, rats were killed at different time points after ischemia or sham operation. The ipsilateral hemisphere was harvested, homogenized in cold lysis buffer (1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 50 mmol/L Tris, 1% NP-40, pH 7.5, 5 mmol/L EDTA, 1% sodium deoxycholate, 1 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mmol/L PMSF) and centrifuged at 14,000 rpm and 4°C for 15 min to collect the supernatant. Protein concentrations were determined using BCA protein assay, (KangChen, KC-430, China). Protein samples were subjected to SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidine diflouride filter membrane. Membranes were blocked with 5% BSA for one hour, incubated with primary antibody against FOXJ1 (anti-mouse, 1:3,000; Millipore) at 4°C overnight, washed three times with 0.1% Tween-20 in Tris Buffered Saline (TBS), and incubated with 1:5000 secondary antibody (Kangchen) in 5% BSA, 0.1% Tween-20 in TBS for one hour. Enhanced chemiluminescence reagent (KangChen, KC-420) and film were used for detection. Densitometric analysis of bands was performed using Image J software. Anti-GAPDH antibody was used to verify equal loading.

Sections and Immunohistochemistry

Animals were intraperitoneally injected with chloral hydrate and transcardially perfused with 4% paraformaldehyde (PFA) at Day 1 and Day 3 after ischemia or sham operation, respectively. Brains were removed, post fixed in PFA, embedded in paraffin. Then, 10-μm coronal sections were prepared and examined. Sections were deparaffiniged, boiled in 0.2% citrate buffer and incubated with blocking solution (0.3% Triton X-100; 10% normal goat serum in phosphate buffered saline (PBS); one hour (h), RT) for two hours at room temperature, then incubated with anti-FOXJ1 antibody (anti-mouse, 1:100, millipore) overnight at 4°C. After incubation with the biotinylated goat anti-mouse IgG and SABC as the second antibody for 30 minutes (min), respectively, at 37°C, the reaction sections were incubated with the liquid mixture (0.02% daminobenzidine tetrahy-drochloride,

https://doi.org/10.1017/S0317167100014372 Published online by Cambridge University Press

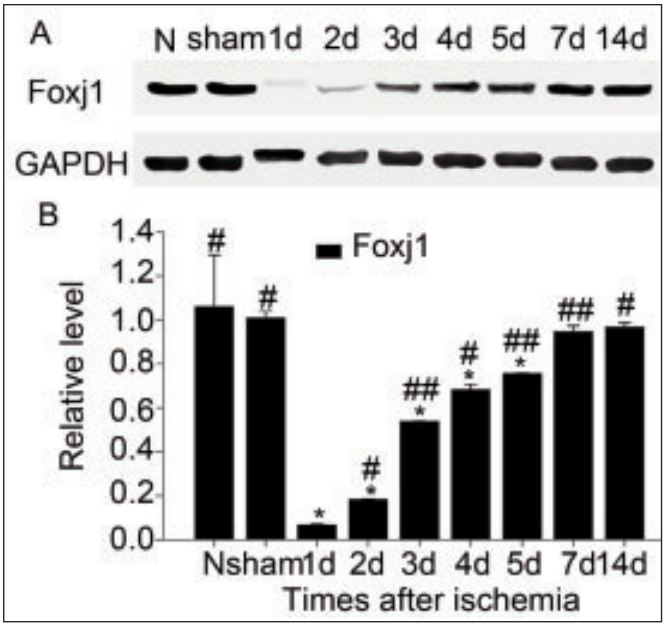

Figure 1: *FOXJ1 expression in the ischemic hemisphere assessed by Western blot.* ***A.*** *FOXJ1 protein level was relatively lower in MCAO rats, but gradually increased.* ***B.*** *Semiquantitative analysis (relative optical density) showed the intensity of staining of FOXJ1 to GAPDH for each time point. The data were means ± SEM (n =6, *P < 0.05 compared with sham; #P< 0.05 compared with Day 1 MCAO; ##P<0.01 compared with Day 1 MCAO.*

3% H_2O_2, and 0.1% PBS). Finally, the sections were dehydrated and covered with coverslips. We examined the sections and counted the cells with strong or moderate brown staining, weak or no staining as positive or negative FOXJ1 cells, respectively. From each group at higher magnified images, the average values of each group were calculated.

Double Immunofluorescent Staining

Sections were deparaffiniged, boiled in 0.01 M Folic acid repair liquid for 15 minutes for antigen retrieval. We blocked the sections with normal goat serum for 20 minutes at 37°C, incubated them with rabbit polyclonal primary antibodies for FITC-conjugated anti -MAP-2 (a marker of neurons, 1:200; Bioss), and FITC-conjugated anti-GFAP (a marker of astrocytes, 1:300; Bioss), and FITC-conjugated anti–NeuN (a marker of neurons, and FITC-conjugated anti-FOXJ1 (1:200; Bioss), 1:200; Bioss), and Cy3-conjugated anti-Ki67 (a marker of cell proliferation, 1: 200; Bioss), and Cy3-conjugated anti–FOXJ1 (1:200; Bioss) over night at 4°C. Then sections were incubated with secondary antibodies for 90 minutes at 37°C. The stained sections were examined with a Leica fluorescencemicroscope (Germany).

Quantitative Analysis

Cells double labeled for FOXJ1 and the other phenotypic markers used in the experiment were quantified. Sections were double labeled for FOXJ1 and MAP-2 and GFAP. To identify the number of each phenotype-specific marker-positive cells expressing FOXJ1, phenotype-specific marker-positive cells were counted in each section. Then double labeled cells for FOXJ1 and phenotype-specific markers were recorded. Six adjacent sections per animal were sampled.

Statistical Analysis

All data were analyzed with SPSS 17.0 statistical software. All values were expressed as mean ± SEM. Student t test was used when only two groups were compared. One-way analysis of variance followed by the Tukey's post hoc multiple comparison tests was used for statistical analysis. P values less than 0.05 were considered statistically significant.

Results

The Expression of FOXJ1 in the Ischemic Hemisphere by Western Blot

In order to investigate the temporal patterns of FOXJ1 expression after transient focal cerebral ischemia, Western blot was performed in this study. As shown in Figure 1, in the MCAO rats, the expression of FOXJ1 was reduced significantly after operation compared to sham group and normal rats. Its expression gradually increased from Day 1 to Day 14 after ischemia, the level of FOXJ1 at Day 1 to Day 3 was significantly lower than the other time points. The level of FOXJ1 at 14 days was same as sham group. There was no significant difference between normal and sham group.

The Changed Distribution of FOXJ1 in the Brain After Cerebral Ischemia

We used the coronal sections from the sham-operation, Day1 and Day 3 after ischemia to assess the changed distribution of the expression of FOXJ1 by immunohistochemistry. We found that FOXJ1 was expressed in the whole sham-operated brain (Figure 2C, F, I, L). There was no positive immunostaining in the ischemic core (IC) (Figure 2D, E), but the immunostaining of FOXJ1 deposited strongly in the ipsilateral and contralateral hemisphere in the cortical penumbra (CP) (Figure 2G, H, J, K). We noticed there were more FOXJ1 staining positive cells in the IC than cortical penumbra, and most FOXJ1 staining positive cells were located on peripheral area of ischemic core (Figure 2A, B). In addition, the staining of FOXJ1 was significantly increased in the cortical penumbra (CP) at Day 3 than Day 1. However, there was no significant difference between the ipsilateral and contralateral hemispheres (Figure 2M).

The Colocalization of FOXJ1 with Different Cellular Markers by Double Immunofluorescent Staining

To further identify the cell types expressing FOXJ1 in the cortical penumbra after transient focal cerebral ischemia, we used double immunofluorescent staining with MAP-2 (a marker of neurons) and GFAP (a marker of astrocytes). We found that FOXJ1 was expressed in astrocytes (Figure 3A-C) and neurons (Figure 3J-L) in Day 1 brain and with a relatively high level in brain of Day 3 (Figure 3D-F, M-O). To identify the number of each phenotype-specific marker-positive cells expressing FOXJ1, phenotype specific marker-positive cells were counted between Day 1 and Day 3 after transient focal cerebral ischemia

https://doi.org/10.1017/S0317167100014372 Published online by Cambridge University Press

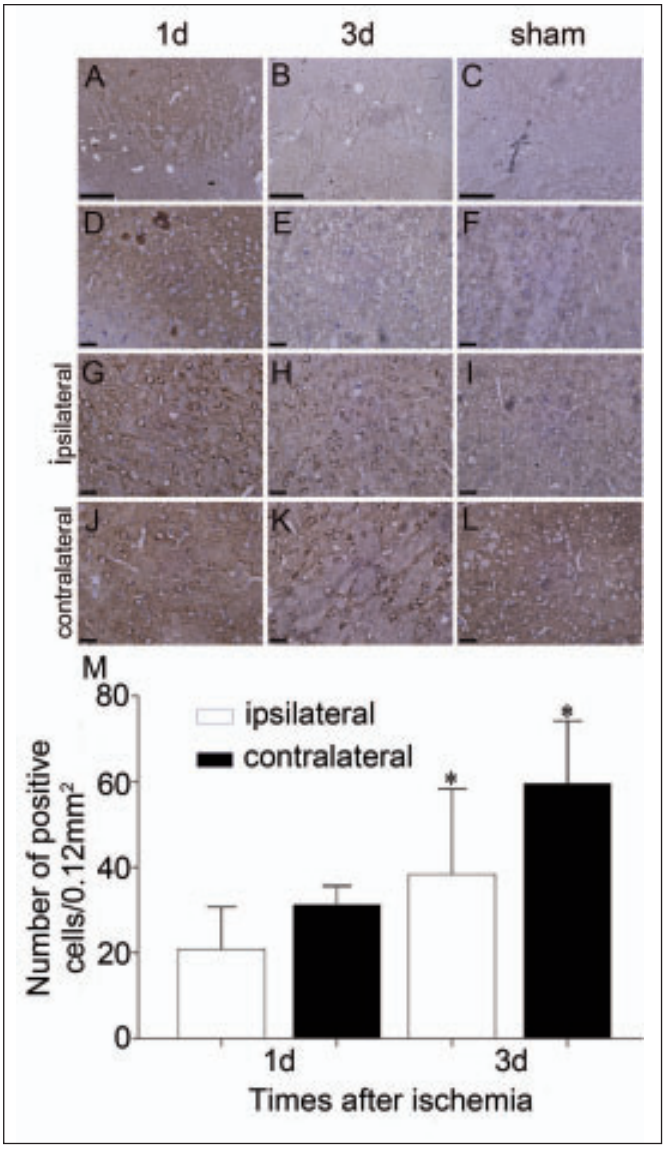

Figure 2: *The changed distribution of FOXJ1 in the brain after cerebral ischemia.* ***A,B.*** *The positive cells in the cortical penumbra migrated to the ischemic core.* ***C.*** *Sham-operated brain had no cell migration.* ***D,E.*** *There was no positive immunostaining in the ischemic core.* ***F.*** *There was positive immunostaining in the same site in the sham-operated brain.* ***G,H,J,K.*** *The immunostaining of FOXJ1 deposited strongly in the ipsilateral and contralateral hemisphere in the cortical penumbra.* ***I,L.*** *Immunostaining of FOXJ1 in the sham-operared brain.* ***M.*** *Quantitative analysis of FOXJ1 positive cells/0.12mm² between Day 1 and Day 3 in the contralateral and ipsilateral brains after ischemia. FOXJ1 was significantly increased at three days after ischemia in both contralateral and ipsilateral brains, there was no significant difference between ipsilateral and contralateral hemisphere (*P < 0.05 compared with 1 day). Error bars SEM. Scale bars:* ***A-C*** *80 μm;* ***D-L*** *20 μm.*

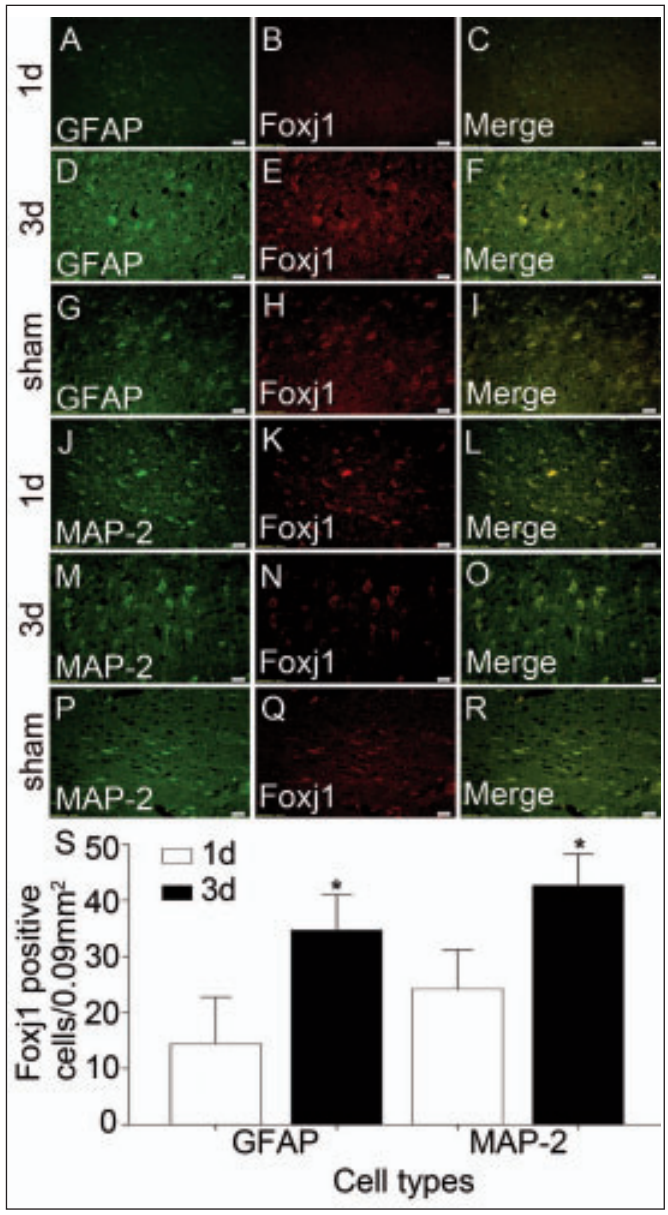

Figure 3: *Double immunofluorescence staining for FOXJ1 and different phenotype-specific markers in adult rat brain Day 1 and Day 3 after transient focal cerebral ischemia. The pictures were taken from ischemic peripheral penumbra area. The sections from sham and ischemic brains Day 1 and Day 3 after transient focal cerebral ischemia were immunostained with FOXJ1 (red,* ***B, E, H , K , N, Q****) and different cell markers, such as MAP-2 (a marker of neurons, green,* ***A, D, G****) and GFAP (a marker of astrocytes, green,* ***J, M, P****), and the colocalization of FOXJ1 with different phenotype-specific markers were visualized in the merged images (****C, F, I, L, O, R****).* ***A-C****,* ***J-L*** *Immunostaining for FOXJ1 with MAP-2 and GFAP at Day 1 after transient focal cerebral ischemia;* ***D-F****,* ***M-O*** *Immunostaining for FOXJ1 with MAP-2 and GFAP at Day 3 after transient focal cerebral ischemia;* ***G-L****,* ***P-R*** *Immunostaining for FOXJ1 with MAP-2 and GFAP in sham brains. Quantitative analysis of different phenotype-specific markers in positive cells expressing FOXJ1 in Day 1 and Day 3 after ischemia. The change of FOXJ1 was significant in neurons and astrocytes; *P <0.05 compared with the 1 day group. Error bars SEM. Scale bars 20μm (****A -R****)*

https://doi.org/10.1017/S0317167100014372 Published online by Cambridge University Press

in each section (Figure 3P). After ischemia, FOXJ1 expression was increased significantly in astrocytes and neurons at Day 3 compared with Day 1.

The Relationship Between FOXJ1 and Cellular Proliferation in Rat Brain after Transient Focal Cerebral Ischemia

In order to describe the proliferative cell types in the cortical penumbra after transient focal cerebral ischemia, we performed double immunofluorescent staining with NeuN (a marker of neurons), GFAP (a marker of astrocytes), and Ki67 (a marker of cell proliferation) in the brain of Day 3 after ischemia. Then we analyzed their relationship between FOXJ1 and these markers. We could find that there were colocalizations between FOXJ1 and Ki67 (Figure 4A-C), NeuN and Ki67 (Figure 4D-F), GFAP and Ki67 (Figure 4G-I). It proved that neurons and astrocytes proliferated after ischemia and FOXJ1 were related to cell proliferation.

Discussion

Stroke is the second commonest cause of death and the leading cause of adult disability in China[23]. Cerebral ischemia is a multifactorial disorder which includes a number of pathways for progression of brain cell injury. Activation of glutamate receptors, release of nitric oxide, proteases and generation of free radicals are important mechanisms in ischemia. However, we found that natural replacement of neurons and synapses might contribute to functional recovery after cerebral ischemia. Stem cells give rise to other stem cells as well as progenitor cells. Intrinsic stem cells in the adult hippocampus generate neurons that integrate into the existing neuronal network. Progenitor cells divide into neurons, astrocytes, and oligodendrocytes. Cerebral ischemia enhances proliferation of neuronal precursor cells in the SVZ, followed by their migration into ischemic brain regions, where they differentiate and mature[24]. In addition, ectopic neurogenesis has been observed in animal models in the ipsilateral striatum of MCA occlusion[25,26] and in degenerated hippocampal CA1 with global cerebral ischemia[27,28]. In our research, we clearly found that neurogenesis existed in the brain after transient focal cerebral ischemia. There were new-born neurons in the cortical penumbra and they migrated to the ischemic core after ischemia.

FOXJ1 is a forkhead transcription factor expressed in multiple tissues during development and is a major regulator of cilia development. FOXJ1 is associated with the production of motile cilia that are several times the length of primary cilia[11,12,29,30].

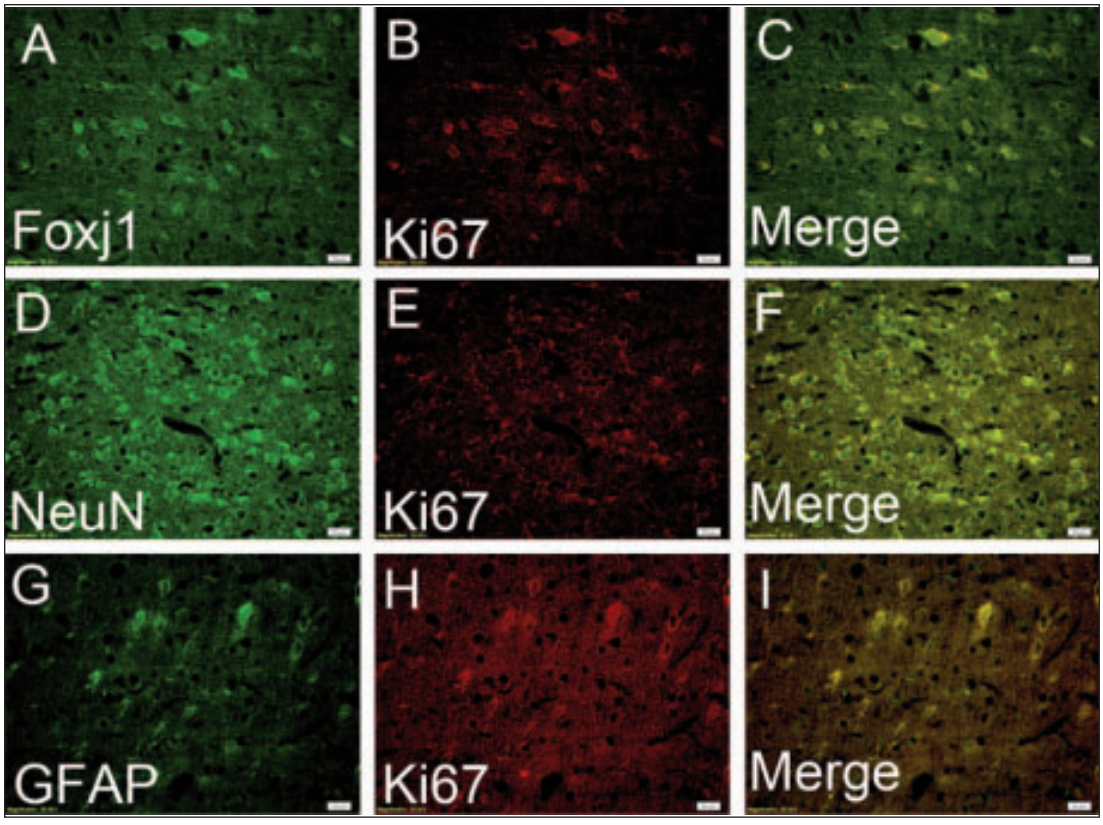

Figure 4: *Double immunofluorescence staining for cellular proliferation in the brains after transient focal cerebral ischemia. Double immunofluorescence staining for NeuN (a marker of neurons, green, d), GFAP (green, **G**), FOXJ1 (green, **A**), and Ki67 (a marker of cell proliferation, red, **B, E, H**) cortical penumbra after transient focal cerebral ischemia. In the rat brain of Day 3 after ischemia, there were co-localization between FOXJ1 and Ki67 (**A-C**), NeuN and Ki67 (**D-F**), GFAP and Ki67 (**G-I**). Scale bars 20μm (**A-I**).*

https://doi.org/10.1017/S0317167100014372 Published online by Cambridge University Press

Motile cilia are important elements of ependymal cell differentiation and function in the central nervous system[13,14]. Neurogenesis persists in the adult mammalian brain in mainly two restricted areas, the SVZ of the lateral ventricles and the detate gyrus of the hippocampus[6]. Stroke can induce a marked increase in cell proliferation in SVZ; these cells migrate into the damaged area of striatum where they express markers of striatal medium spiny neurons[25]. FOXJ1-dependent secreted factors from FOXJ1 cells influence both the proliferation and differentiation potential of progenitors in the embryonic LGE and OVZ, as well as those in the subependymal zone (SEZ), rostral migratory stream (RMS), and olfactory bulb (OB)[19].

Recent studies demonstrated that ependymal cells were quiescent and did not contribute to neurogenesis under normal conditions by genetic fate mapping. However, in response to a stroke, which causes extensive damage to striatum and cerebral cortex, ependymal cells delaminated to the subventricular zone, lost ependymal cell features, entered the cell cycle and gave rise to neuroblasts and astrocytes[17]. Recent studies found that FOXJ1 was required for postnatal differentiation of ependymal cells and a subset of astrocytes in the SVZ, where these cells formed a postnatal neural stem cell niche[18]. The findings of the studies revealed that the subset of astrocytes harvested from the SVZ generate neurospheres, which had the capability of self-renew and had the potential to give rise to neurons, astrocytes, and oligodendrocytes, thus functionally resembling adult neural stem cells. Recent studies have revealed that FOXJ1 promoter-active cells in the spinal canal and SVZ participate in neurogenesis and gliogenesis in spinal cord injury and in response to stroke[16,17].

In the current study, we displayed the increased expression of FOXJ1 in adult rat brain after transient focal cerebral ischemia. We found that the expression of FOXJ1 was gradually increasing and had a significant growth at Day 3 in the 14 days after ischemia by Western blot analysis. The expression after ischemia was lower than normal and sham-operated brain, but the level of FOXJ1 at 14 days was the same as normal brain. Immuohistochemical staining told us that the immunostaining of FOXJ1 deposited strongly in the ipsilateral and contralateral hemisphere in the cortical penumbra (CP). In addition, the staining of FOXJ1 was significantly increased in the CP at Day 3 compared to Day 1. There is no FOXJ1 expression in the ischemic core (IC). We speculated that new-born FOXJ1 cells must have occured, although many cells were dead, due to apoptosis and necrosis induced by ischemia. By double immunofluorescent staining we observed that there was colocalization of FOXJ1 and MAP-2, as well as FOXJ1 and GFAP in CP. In addition, colocalization of FOXJ1 with MAP-2 and GFAP was increased in the brain Day 3 after ischemia compared to Day 1.

Stroke results in increased neuroblast formation in the adult ventricle wall and generation of mature striatal neurons and also induces ependymal cells to regain radial glial features[31]. Parent et al[32] demonstrated that SVZ neurogenesis increased from Day 10 to day 21 after MCAO for 90 minutes, and chains of neuroblasts extended from SVZ to the peri-infarct striatum in rats. Similar findings were reported by Ahmed et al[33]. Jin et al[34] observed SVZ neurogenesis for up to two weeks after MCAO for 90 minutes, and report that neural progenitor cells migrate into the ischemic penumbra of the adjacent striatum and also into the penumbra of ischemic cortex via the rostral migratory and lateral cortical streams. There is also evidence that induction of endogenous cell proliferation and neurogenesis after cerebral ischemia is not restricted to the ipsilateral hippocampal formation, but can also be found in the contralateral hemisphere[35]. In our experiment, we observed the new-born neurons and glial cells in the contralateral hemisphere. Besides this evidence, the biological relevance of ischemia-induced endogenous neurogenesis is still elusive considering that the majority of new-born cells die within weeks after the onset of ischemia[36,37]. Therefore, enhanced postischemic survival rates of new-born neural precursor cells are necessary, if endogenous neurogenesis after stroke is to yield functional benefits. Our study did not clearly explain the role of FOXJ1 after ischemia in the long term and this needs further study.

Neurogenesis makes the most important contribution in functional recovery after cerebral ischemia. Our study revealed that the expression of FOXJ1 was gradually increased in the rat brain after ischemia, which supported the concept that FOXJ1 may play an important role in the physiological and the pathological process in the ischemic brain. Our results suggest that FOXJ1 may be required for the differentiation of the cells acting as adult neural stem cells which participate in neurogenesis and give rise to neurons, astrocytes, and oligodendrocytes. These cells may migrate to the lesion region by stimulation of ischemia to compensate for the loss of neuronal function caused by ischemia. In the current study, we found there were more FOXJ1 staining positive cells in the ischemic core than cortical penumbra, and most FOXJ1 staining positive cells were located in the peripheral area of the ischemic core, so we hypothesized that positive cells in the cortical penumbra migrated to the ischemic core, which was also reported in a previous study[38]. FOXJ1 can become a new target and objective in the study of neurological protection and functional recovery in the field of neurogenesis after cerebral ischemia. Further studies are needed to confirm the inherent mechanisms of the role of FOXJ1 after cerebral ischemia

Acknowledgment

This work was supported by Program of scientific innovation research for college graduate in Jangsu province, Grant # 22110124.

References

1. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008 May 10;371(9624):1612-23.
2. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. Glia. 2005 Jun;50(4):427-34.
3. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol. 2007 Mar;184(1-2):53-68.
4. del Zoppo GJ, Milner R, Mabuchi T, et al. Microglial activation and matrix protease generation during focal cerebral ischemia. Stroke. 2007 Feb;38(2 Suppl):646-51.
5. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 2009 Dec;32(12):638-47.
6. Gage FH. Mammalian neural stem cells. Science. 2000 Feb 25;287 (5457):1433-8.
7. Lichtenwalner RJ, Parent JM. Adult neurogenesis and the ischemic forebrain. J Cereb Blood Flow Metab. 2006 Jan;26(1):1-20.
8. Clevidence DE, Overdier DG, Tao W, et al. Identification of nine tissue-specific transcription factors of the hepatocyte nuclear factor 3/forkhead DNA-binding-domain family. Proc Natl Acad Sci USA. 1993 May 1;90(9):3948-52.
9. Hackett BP, Brody SL, Liang M, Zeitz ID, Bruns LA, Gitlin JD. Primary structure of hepatocyte nuclear factor/forkhead homologue 4 and characterization of gene expression in the developing respiratory and reproductive epithelium. Proc Natl Acad Sci USA. 1995 May 9;92(10):4249-53.
10. Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD. Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am J Respir Cell Mol Biol. 2000 Jul;23(1):45-51.
11. Yu X, Ng CP, Habacher H, Roy S. Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat Genet. 2008 Dec;40(12):1445-53.
12. Stubbs JL, Oishi I, Izpisua Belmonte JC, Kintner C. The forkhead protein Foxj1 specifies node-like cilia in Xenopus and zebrafish embryos. Nat Genet. 2008 Dec;40(12):1454-60.
13. Spassky N, Merkle FT, Flames N, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. J Neurosci. 2005 Jan 5;25(1):10-8.
14. Sawamoto K, Wichterle H, Gonzalez-Perez O, et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. Science. 2006 Feb 3;311(5761):629-32.
15. Cui G, Yu Z, Li Z, et al. Increased expression of Foxj1 after traumatic brain injury. J Mol Neurosci. 2011 Oct;45(2):145-53.
16. Meletis K, Barnabe-Heider F, Carlen M, et al. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol. 2008 Jul 22;6(7):e182.
17. Carlen M, Meletis K, Goritz C, et al. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci. 2009 Mar;12(3):259-67.
18. Jacquet BV, Salinas-Mondragon R, Liang H, et al. FoxJ1-dependent gene expression is required for differentiation of radial glia into ependymal cells and a subset of astrocytes in the postnatal brain. Development. 2009 Dec;136(23):4021-31.
19. Jacquet BV, Muthusamy N, Sommerville LJ, et al. Specification of a Foxj1-dependent lineage in the forebrain is required for embryonic-to-postnatal transition of neurogenesis in the olfactory bulb. J Neurosci. 2011 Jun 22;31(25):9368-82.
20. Belayev L, Alonso OF, Busto R, Zhao W, Ginsberg MD. Middle cerebral artery occlusion in the rat by intraluminal suture. Neurological and pathological evaluation of an improved model. Stroke. 1996 Sep;27(9):1616-22; discussion 23.
21. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989 Jan;20(1):84-91.
22. Takemura S, Kayama T, Kuge A, et al. Correlation between copper/zinc superoxide dismutase and the proliferation of neural stem cells in aging and following focal cerebral ischemia. J Neurosurg. 2006 Jan;104(1):129-36.
23. Bonita R, Mendis S, Truelsen T, Bogousslavsky J, Toole J, Yatsu F. The global stroke initiative. Lancet Neurol. 2004 Jul;3(7):391-3.
24. Jin K, Minami M, Lan JQ, et al. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA. 2001 Apr 10;98(8):4710-5.
25. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med. 2002 Sep;8(9):963-70.
26. Zhang R, Wang Y, Zhang L, et al. Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats. Stroke. 2002 Nov;33(11):2675-80.
27. Nakatomi H, Kuriu T, Okabe S, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell. 2002 Aug 23;110(4): 429-41.
28. Bendel O, Bueters T, von Euler M, Ove Ogren S, Sandin J, von Euler G. Reappearance of hippocampal CA1 neurons after ischemia is associated with recovery of learning and memory. J Cereb Blood Flow Metab. 2005 Dec;25(12):1586-95.
29. Blatt EN, Yan XH, Wuerffel MK, Hamilos DL, Brody SL. Forkhead transcription factor HFH-4 expression is temporally related to ciliogenesis. Am J Respir Cell Mol Biol. 1999 Aug;21(2):168-76.
30. Tichelaar JW, Lim L, Costa RH, Whitsett JA. HNF-3/forkhead homologue-4 influences lung morphogenesis and respiratory epithelial cell differentiation in vivo. Dev Biol. 1999 Sep 15;213 (2):405-17.
31. Zhang RL, Zhang ZG, Wang Y, et al. Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain. J Cereb Blood Flow Metab. 2007 Jun;27(6): 1201-12.
32. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997 May 15;17(10): 3727-38.
33. Ahmed S, Reynolds BA, Weiss S. BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. J Neurosci. 1995 Aug;15(8):5765-78.
34. Jin K, Mao XO, Sun Y, Xie L, Greenberg DA. Stem cell factor stimulates neurogenesis in vitro and in vivo. J Clin Invest. 2002 Aug;110(3):311-9.
35. Takasawa K, Kitagawa K, Yagita Y, et al. Increased proliferation of neural progenitor cells but reduced survival of newborn cells in the contralateral hippocampus after focal cerebral ischemia in rats. J Cereb Blood Flow Metab. 2002 Mar;22(3):299-307.
36. Haas S, Weidner N, Winkler J. Adult stem cell therapy in stroke. Curr Opin Neurol. 2005 Feb;18(1):59-64.
37. Parent JM. Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist. 2003 Aug;9(4):261-72.
38. Jin L, Sliz P, Chen L, et al. An asymmetric NFAT1 dimer on a pseudo-palindromic kappa B-like DNA site. Nat Struct Biol. 2003 Oct;10(10):807-11.