Brain Research 1648 (2016) 163–171

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Increased expression of suppressor of cytokine signaling 2 in the subventricular zone after transient focal cerebral ischemia in adult rats

Yoo-Jin Shin*, Tae-Ryong Riew, Xuyan Jin, Jeong-Heon Choi, Mun-Yong Lee

Department of Anatomy, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form
20 July 2016
Accepted 25 July 2016
Available online 27 July 2016

Keywords:
Suppressor of cytokine signaling 2
Neural stem cells
Immature neurons
Focal ischemia
Subventricular zone

ABSTRACT

Suppressor of cytokine signaling 2 (SOCS2) is a well-established negative regulator of growth hormone signaling that acts on adult hippocampal neurogenesis during ischemic insults. To explore whether SCOS2 is involved in poststroke neurogenesis, we studied the temporal expression of *SOCS2* mRNA in the subventricular zone (SVZ) of rats after transient focal cerebral ischemia. We found that *SOCS2* expression was upregulated in the SVZ of the infarcted hemisphere. The number of *SOCS2*-expressing cells was significantly increased in the ipsilateral SVZ compared with that on the contralateral side on days 7–10 after reperfusion, and *SOCS2*-expressing cells were highly proliferative, coinciding both spatially and temporally with stroke-induced neurogenesis. Almost all *SOCS2*-expressing cells in the SVZ were colabeled with the neural stem cell markers nestin and musashi1 and the neural/glial progenitor transcription factor Sox-2. In addition, *SOCS2* was highly expressed in newly generated neurons that were immunoreactive for polysialic acid-neural cell adhesion molecule, indicating that *SOCS2* expression may be persistent during neuronal differentiation. Thus, our data demonstrated that *SOCS2* mRNA was highly expressed in proliferating neural stem/precursor cells and postmitotic migratory neuroblasts in the SVZ niche after focal cerebral ischemia, suggesting that *SOCS2* may be actively involved in regulating adult neurogenesis induced by ischemic stroke.

1. Introduction

The suppressor of cytokine signaling (SOCS) family, including SOCS1–7 and cytokine-inducible Src homology 2 protein, plays a unique role in downregulating cytokine signaling pathways and thereby controlling the cellular response to several cytokines and growth factors (Campbell, 2005; Gregorieff et al., 2000; Hilton et al., 1998; Kile and Alexander, 2001; Krebs and Hilton, 2000; Starr et al., 1997). Among SOCS family members, SOCS2 is primarily characterized as a negative regulator of the growth hormone/insulin-like growth factor-1 signaling pathway, but also has important roles in the regulation of diverse cellular processes in health and various pathologies (Letellier and Haan, 2016; Rico-Bautista et al., 2006). In particular, SOCS2 has been extensively studied in the central nervous system (CNS) and is actively involved in neural development, growth, and differentiation (Polizzotto et al., 2000; Ransome and Turnley, 2005; Turnley et al., 2002). SOCS2 is also able to regulate neurite outgrowth and length (Goldshmit et al., 2004a, 2004b; Scott et al., 2006) and has recently been shown to function as a regulator of neurotrophin receptor TrkA signaling (Uren et al., 2014; Uren and Turnley, 2014). In addition to its role in neurogenesis during development, Ransome and Turnley (2008) reported that overexpression of SOCS2 enhances basal and exercise-induced hippocampal neurogenesis, suggesting that SOCS2 may act as an important regulator in adult hippocampal neurogenesis.

Despite extensive research, few reports have described the role of SOCS2in the diseased CNS. Interestingly, SOCS2expression is induced in reactive astrocytes and is transiently increased in neural progenitor cells/immature neurons in the subgranular zone (a well-recognized germinal center) in the ischemic hippocampus, suggesting that SOCS2may be involved in the glial reaction and the regulation of adult hippocampal neurogenesis in ischemic brains (Choi et al., 2009). Thus, these results led us to hypothesize that SOCS expression may be regulated in the subventricular zone (SVZ) of the lateral ventricle, another neurogenic niche, by transient focal cerebral ischemia because this insult enhances SVZ neurogenesis and because SVZ-derived neural stem cells have the potential to migrate toward the peri-infarct and infarct area (Arvidsson et al., 2002; Goings et al., 2004; Jin et al., 2002; Jin et al., 2003; Parent et al., 2002; Zhang et al., 2001, 2014). However, whether SOCS2 plays a role in SVZ neurogenesis after ischemic insults has not yet been determined.

In the present study, we examined the temporal regulation of

* Correspondence to: Department of Anatomy, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu Seoul 137-701, Republic of Korea.

http://dx.doi.org/10.1016/j.brainres.2016.07.040
0006-8993/

SOCS2 mRNA expression in the SVZ of adult rats after focal cerebral ischemia by *in situ* hybridization analysis. We also investigated the phenotypes of cells expressing *SOCS2* in the SVZ niche using double- and triple-labeling techniques. Finally, we evaluated the expression of *SOCS2* mRNA in proliferating precursor cells by 5-bromo-2′-deoxyuridine (BrdU) incorporation in this model.

2. Results

2.1. SOCS2 mRNA expression was upregulated in the ipsilateral SVZ after reperfusion

Consistent with a previous study (Shin et al., 2010), cresyl violet-stained sections revealed that the 60-min middle cerebral artery occlusion (MCAO) caused cerebral infarction, which was confined to the ipsilateral MCA territory, including the majority of the striatum and cerebral cortex, but not extended into the SVZ of the lateral ventricle (data not shown). For assessment of morphological alterations in the SVZ after ischemic stroke, we performed nestin immunohistochemistry in sham-operated rats and rats reperfused for 7 days after MCAO. Expansion of the ipsilateral SVZ after MCAO was observed in comparison with that of sham-operated rats and the corresponding contralateral sides (Suppl. Figs. S1C–H).

After inducing a stroke, we analyzed the temporal profile of *SOCS2* mRNA expression using *in situ* hybridization in the SVZ niche. In sham-operated rats, weak hybridization signals for *SOCS2* were observed in the SVZs of both ipsilateral and contralateral hemispheres (Fig. 1A, B). Adjacent sections from these animals were routinely processed for *in situ* hybridization with the *SOCS2* sense probe, and no specific cellular labeling was observed (Suppl. Figs. S1A, B). In animals reperfused for 7–14 days after MCAO, *SOCS2* expression appeared to be increased throughout the SVZ of the ipsilateral hemisphere (Fig. 1D, F, H) as compared with the sham-operated control animals (Fig. 1A, B) and the corresponding contralateral (i.e., nonischemic) hemisphere (Fig. 1C, E, G). Image analysis revealed that the mean fluorescence intensity of *SOCS2* expression in the ipsilateral SVZ was significantly increased compared with that of the corresponding contralateral SVZ at days 10 and 14 after reperfusion (Fig. 1I).

2.2. SOCS2 mRNA was expressed in neural stem/progenitor cells in the SVZ ipsilateral to the MCAO insults

As described above, *SOCS2* expression was upregulated in the ipsilateral SVZ on days 7–14 after reperfusion, during which focal ischemia-induced cell proliferation in the SVZ is most prominent (Ohab and Carmichael, 2008; Yamashita et al., 2006). In order to

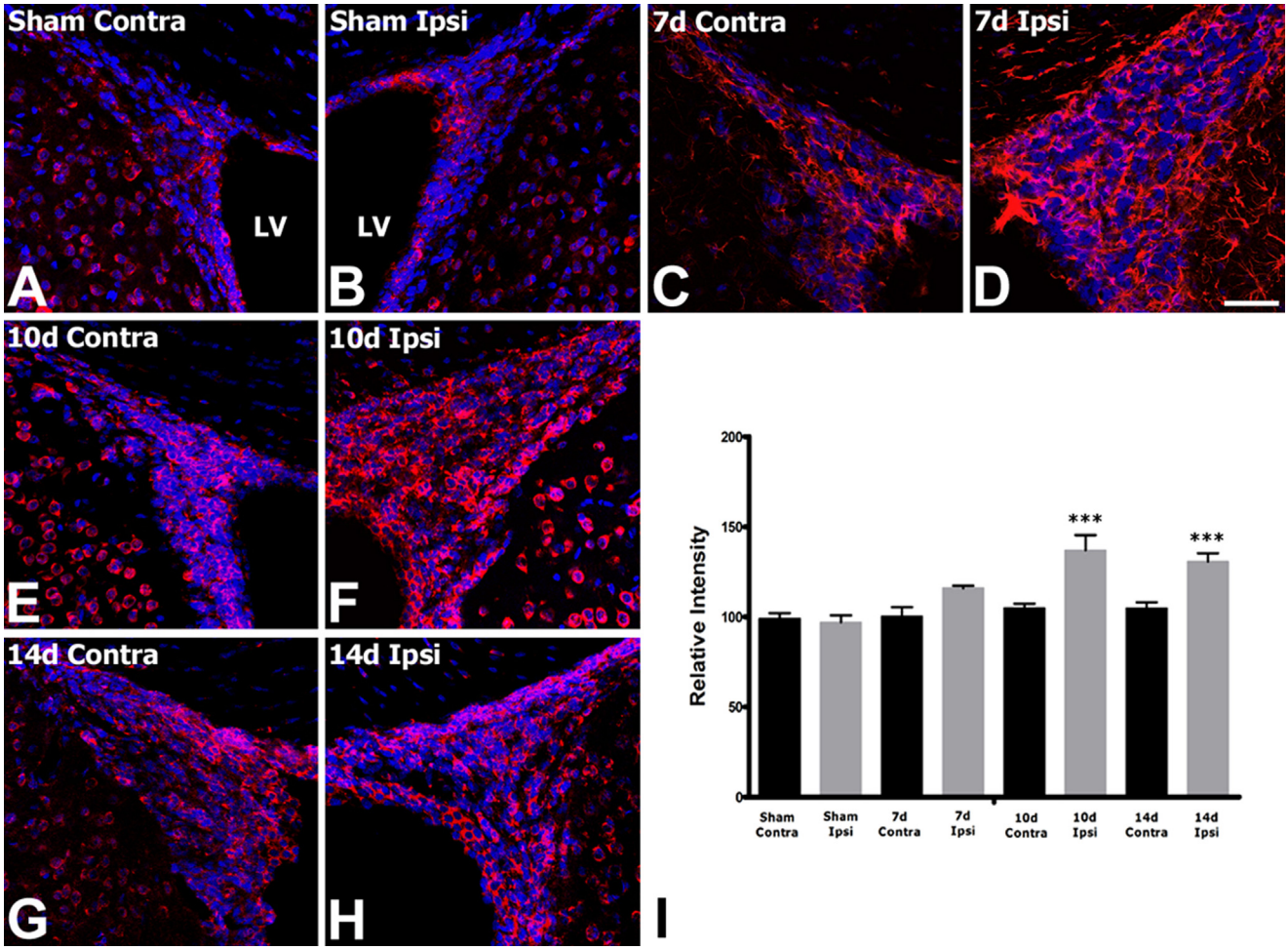

Fig. 1. Changes in *SOCS2* mRNA expression in the dorsolateral subventricular zone (SVZ) of the lateral ventricle (LV) after focal cerebral ischemia. (A, B) In sections from sham-operated animals, weak hybridization signals of *SOCS2* were observed in the SVZ of both contralateral (A) and ipsilateral hemispheres (B). (C–H) The labeling intensity of *SOCS2* mRNA was markedly increased in the dorsolateral SVZ of the ipsilateral hemisphere on days 7 (D) and 10 (F) after reperfusion and was sustained until 14 days (H) compared with the corresponding contralateral SVZ (C, E, G). (I) The mean fluorescence intensity of *SOCS2* expression was significantly increased in the ipsilateral SVZ compared with that of the corresponding contralateral SVZ on days 10 and 14 after reperfusion. The data are represented as the mean ± SEM;$^{***}P < 0.001$ compared with the contralateral SVZ. Scale bars = 50 μm for A–H.