Neuroscience Letters 631 (2016) 85–90

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Transgenic over-expression of slit2 enhances disruption of blood-brain barrier and increases cell death after traumatic brain injury in mice

Shuai Li[a,c,1], Hang Li[a,1], Xiao-fei He[b], Ge Li[a], Qun Zhang[d], Feng-ying Liang[b], Huan-huan Jia[a], Jiang-chao Li[c], Ren Huang[a], Zhong Pei[b], Li -jing Wang[c,**], Yu Zhang[a,*]

[a] Guangdong Laboratory Animals Monitoring Institute, Guangdong Provincial Key Laboratory of Laboratory Animals, Guangzhou, China
[b] Department of Neurology, National Key Clinical Department and Key Discipline of Neurology, Guangdong Key Laboratory for Diagnosis and Treatment of Major Neurological Diseases, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
[c] Vascular Biology Research Institute, School of Basic Course, Guangdong Pharmaceutical University, Guangzhou,China
[d] Department of Rehabilitation Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

HIGHLIGHTS

- Two-photon microscopy was used to examine the role of slit2 in micro TBI (traumatic brain injury).
- BBB (Blood Brain Barrier) was damaged in the slit2 over expression mice at the acute stage of micro TBI.
- Cell death was accelerated in the slit2 over expression mice in micro TBI.

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 24 July 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
Silt2
Transgenic mice
Micro traumatic brain injury (TBI)
Blood brain barrier (BBB)
Cell death

ABSTRACT

Traumatic brain injury (TBI) is the leading cause of mortality and disability among male adolescents and young adults; and mild traumatic brain injury is the most common type of traumatic brain injury. The disruption of blood-brain barrier (BBB) plays an important role in brain trauma. Previously, we have found that slit2, a member of slit protein family, increases permeability of BBB. In the present study, we examined the role of slit2 in the pathogenesis of mild TBI in a mouse model of micro TBI. Rhodamine BandPI (PropidiumIodide) staining were used to detect the permeability of BBB and cell death, respectively. The leakage of Rhodamine B and cell death were significantly increased in Slit2-Tg mice than in C57 control mice after micro TBI. The present results suggest that over expression of slit2 plays a detrimental role in the pathophysiology of mild TBI.

1. Introduction

Traumatic brain injury (TBI) is the leading cause of mortality and disability among male adolescents and young adults; and it constitutes a major health and socioeconomic problem throughout the world [1]. Micro traumatic brain injury is the most common type of traumatic brain injury [2]. Micro TBI happens at fighting, traffic accidents, construction, industrial and natural disasters. Unfortunately, it is often overlooked because of lack of immediate severe impact. As a result, the mechanisms underlying micro TBI are poorly understood and no single-agent treatment has been successfully used in clinical setting [3]. Therefore, there is a need to study micro TBI and its mechanism.

Slit2 was originally identified in Drosophila as an extracellular cue that guides axon path finding [4]. Structurally, slits contain leucine-rich repeat regions, EGF-like repeats, a laminin-G-like domain and a C-terminal cystine-knot. Slits belong to slit protein family which includes slit1, slit2 and slit3. Slits are the Roundabout genes encoding the trans membrane receptors [5,6]. The slits are involved in many physiological and pathological processes such as axon guidance at the midlinecentral nervous system,tumor apo-

* Corresponding author at: Guangdong Laboratory Animals Monitoring Institute, 11 Fengxin Rd, Ke-Xue-Cheng, Luogang District, Guangzhou 510663, China.
** Corresponding author at: Vascular Biology Research Institute, Guangdong Pharmaceutical University, No. 280 E Road, Higher Education Mega, Guangzhou 510006, China.
E-mail addresses: wanglijing62@163.com (L.-. Wang), zhangyugzh@hotmail.com (Y. Zhang).
[1] Shuai Li and Hang Li contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2016.08.013

ptosis and the regulation of an giogenesis and ischemic injury [7,8]. Interestingly, slit2 is an important regulator of leukocyte trafficking and inflammation and can inhibit leukocyte migration [9]. It was recently reported that Slit2 is associated with adult neuronal function including a variety of pathophysiological processes [10]. In addition, slit2 has been shown to influence the vascular formation in brain and alter the structure of BBB [11,12].

Given that the blood-brain barrier damage plays an important role in brain trauma, studying the role of slit2 in micro TBI may provide new insights into the pathophysiology of micro TBI. In the present study, we examined the role of Slit2 in the pathogenesis of micro TBI in a closed-skull brain injury model in slit2 transgenic mice.

2. Materials and methods

2.1. Experimental animals

C57BL/6J mice (C57 mice) were purchased form model animal research center of Nanjing university. The slit2 over expression mice (Slit2-Tgmice) were generated by the Institute of Biochemistry and Cell Biology, Shanghai, China. All the experimental protocols were performed according to the guidelines of Sun Yat-sen University home office and were approved by the Animal Ethics Committee of Sun Yat-sen University.

2.2. Antibody and reagents

Rhodamine B (Sigma,USA), PropidiumIodide (PI,Thermofisher), Fluorescein–dextran (Sigma, USA).

2.3. Micro TBI preparation

Mice were anesthetized with 4.2% chloral hydrate. Body temperature was maintained at 37 ± 0.5 °C by using a regulated heating pad. After the animal was placed on the heating pad, skull bone was exposed using sterile scissors. The skull was gently drilled and the surface was kept wet with artificial cerebro spinal fluid. The thickness of drilled skull was carefully monitored to prevent brain damage caused by vibration. The operation was stopped when the skull becomes soft, and the surface of the brain blood vessels clearly visible. A cranial window of 2.0 × 2.0-mm size was thinned on the left somato sensory cortex. Then, the mice was placed in a stereotaxic apparatus as previously described [13] with some modification. A surgical instrument was used to gently press the pliable skull bone downward 0.5 mm for 1 min under stereotactic guidance to promote a constant concavity in the bone. This process was to induce compression injury and resulted in the skull bone collapsing inward towards the surface of the brain and without breaking. After that,the mice was placed in the cage for about 24 h.

2.4. 4 In vivo two-photon imaging

For in vivo two-photon imaging, the mouse was fixed with acustom-fabricated metal frame that held the head with acyanoacrylate and dental cement. After that, the mouse was fixed on the stage of a Leica DM6000 CFS (Leica, Germany). Data acquisition and laser scanning were controlled using Leica LAS AF 2.5 software. For imaging vessel network and counting dead cells, a LeicaNA 0.95, 25 × water-immersion objective was used Fig. 1.

2.5. The assessment of the permeability of the BBB

The mouse chosen for the assessment of BBB integrity was deeply anesthetized. The integrity of the blood–brain barrier (BBB) was examined after TBI as previously described [14]. The blood serum was labeled by intravenously injecting 0.2 ml of 2% (wt/vol) solution of Rhodamine B (Sigma, USA) in saline for visualization of the vessels and disruption of BBB, indicated by dye leakage. The blood vessels in the brain were illuminated with a mode locked Ti:sapphire laser unit set at 860 nm, using a 0.95 NA, 25 × water-immersion objective lens. A stack of 10 images, spanning 100–300 mm below the cortical surface, was acquired during a 60 min period.

2.6. Propidium iodide staining

Low molecular weight compounds are able to pass through intact thinned skull into the meninges and parenchyma to reliably label dead cells by transcranial application of PropidiumIodide (PI,Thermofisher) [13]. Therefore, we visualized cell death to evaluate the evolution of injury in the compression site by incubating the thinned skull with PI (1 mg/ml) in artificial cerebrospinal fluid (aCSF) for 30 min followed by a wash with aCSF. Then the blood serum was labeled by intravenously injecting 0.2 ml of 2-MD, a fluorescein–dextran (Sigma, USA) in saline. This was followed by imaging with two-photon microscopy, using a 0.95 NA, 25× magnification water-immersion objective. Stacks of images were acquired using a step size of 2.0 μm to a depth of 200 μm. Cells labeled by PI exhibiting red fluorescence were dead cells. The total number of PI-positive cells in the focal lesion was calculated in three randomly-selected images. The number of PI-positive cells was calculated using the Image J software.

2.7. Cell death volume calculation

Cell death was detected by PI staining and the volume of cell death was calculated according to published literatures [15,16]. PI was incubated by transcranial application to the compression site for 30 min before fixation. After fixation, brain was sliced into 50 μm thick section. Lesion site with PI staining was imaged with confocal microscopy. Images were captured using a Leica SP5 confocal microscope equippedwith a 25× objective. Images were collected using sequential scanning with the 405-, 488-, and 561-nm lasers to produce combined overlays. Total cell death volume was the sum of PI staining volume.

2.8. Statistical analysis

An unpaired *t*-test was used to examine differences between groups, which were performed with Prism5.0 software (GraphPad). A P-value less than 0.01 was accepted as statistically significant.

3. Results

3.1. The permeability of BBB increased in Slit2-Tg mice post-microTBI

The integrity of BBB was examined before and after micro TBI. There was more leak of the 70-kDa dye in Slit2-Tg mice compared with C57 mice in the normal state. But after micro TBI,the leakage of Rhodamine B was significantly increased in Slit2-Tg mice than in C57 mice after micro TBI (Fig. 2). This implies that over expression of Slit2 increases BBB disruption at the early stage of TBI. More of the Rhodamine B dye leaked in Slit2-Tg mice compared with C57 mice, lead to more serious the destruction of the blood brain barrier in Slit2 TBI group (Fig. 2).