Neuroscience Letters 606 (2015) 194–199

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Long-term neuroglobin expression of human astrocytes following brain trauma

Xiameng Chen[a], Yuan Liu[a], Lin Zhang[b], Peng Zhu[c], Haibiao Zhu[a], Yu Yang[a], Peng Guan[a,*]

[a] Department of Forensic Pathology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, PR China
[b] Laboratory of Molecular Translational Medicine, West China Institute of Women and Children's Health, Key Laboratory of Obstetric & Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, PR China
[c] The People's Procuratorate of Chengdu, Sichuan, PR China

HIGHLIGHTS

- Neuroglobin can be expressed by human astrocytes after brain trauma.
- Human astrocytes does not express Neuroglobin in acute brain trauma.
- Human astrocytes express Neuroglobin after sub-acute/chronic brain trauma.
- Astrocytic neuroglobin expression could last 12 months after brain trauma.

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 23 August 2015
Accepted 3 September 2015
Available online 8 September 2015

Keywords:
Neuroglobin
Astrocyte
TBI
Sub-acute
Chronic

ABSTRACT

Neuroglobin (Ngb), a 17 kDa monomeric protein, was initially described as a vertebrate oxygen-binding heme protein in 2000 and detected in metabolically active organs or cells, like the brain, peripheral nervous system as well as certain endocrine cells. A large array of initial experimental work reported that Ngb displayed a neuron restricted expression pattern in mammalian brains. However, growing evidence indicated astrocytes may also express Ngb under pathological conditions. To address the question whether human astrocytes express Ngb under traumatic insults, we investigated Ngb immuno-reactivity in post-mortem human brain tissues that died of acute, sub-acute and chronic brain trauma, respectively. We observed astrocytic Ngb expression in sub-acute and chronic traumatic brains rather than acute traumatic brains. Strikingly, the Ngb immuno-reactive astrocytes were still strongly detectable in groups that died 12 months after brain trauma. Our findings may imply an unexplored role of Ngb in astrocytes and the involved mechanisms were suggested to be further characterized. Also, therapeutic application of Ngb or Ngb-inducible chemical compounds in neuro-genesis or astrocytic scar forming can be expected.

1. Introduction

Globins are generally small proteins that bind oxygen reversibly through the iron-containing porphyrin ring. Identical to the typical structure of oxygen-binding heme globin, human Ngb shares the same helical three-dimensional fold. More than oxygen-binding, Ngb also plays a role in anti-oxidation and free radical scavenging [1].

In vitro, Ngb over-expression significantly diminished the hypoxia/ischemia-induced oxidative damage in cultured neurons [2]. Consistently, the in vivo study revealed prominent reduction of 3-nitrotyrosine (3-NT) production (a common marker for oxidative damage), smaller lesion volumes and improved sensomotor outcomes after traumatic injury in transgenic Ngb over-expressing mice compared with wild type controls [3,4]. In human genetic analysis, Ngb polymorphisms were demonstrated to positively affect the recovery of traumatic brain injury (TBI) patients [5]. Paradoxically, recent research exhibited a reduced infarct size even in Ngb-null mice compared with the wild type controls [6], indicating an intricate role of Ngb. Except for hypoxic/ischemic injuries, increased Ngb expression was also detected in various neurological disorders, such as senility [7], neuronal degeneration [8,9] and

* Corresponding author at: Department of Forensic Pathology, West China School of Preclinical and Forensic Medicine, Sichuan University, 610041 Chengdu, Sichuan, PR China.
E-mail address: guanpengcn@hotmail.com (P. Guan).

http://dx.doi.org/10.1016/j.neulet.2015.09.002
0304-3940/

cancer [10]. Still, the complex functions of Ngb and the underlying mechanisms are poorly characterized and in need of further investigation.

Localization often plays an indicative role in function exploring, therefore, the distribution of Ngb needs to be clearly clarified. Ngb has been consistently detected in various taxa including animals, bacteria and fungi. In vertebrate nervous system, the expression sites of Ngb are both central and peripheral [3,11–11–13], and it is initially expressed in the cerebral cortex and limbic system [12]. In adult brain, Ngb has a wide distribution including cerebellum, olfactory bulb, thalamus, hypothalamus, hippocampus and cerebral cortex [14–16]. However, the cellular localization of Ngb still remains debatable. A couple of research groups reported that Ngb immuno-reactivity was restricted to cytoplasm of neurons [16,17] but not astrocytes, either in normal mouse brain [18] or exposed to hypoxia [19]. Recently, Chen et al. reported detection of Ngb in primary cultured cortical astrocytes of mouse brain [20]. In addition, Lechauve et al. [21] demonstrated the presence of Ngb in astrocytes of optic nerve under physiological conditions. Also, in our previous work, Ngb was detected in human astrocytoma tissues as well as astrocytoma cell lines of both rat and human via quantitative polymerase chain reaction and immuno methods [22]. These results indicated a more complicated expression pattern of Ngb in pathological environment.

One published report which relates to our present work is that Jin et al. failed to detect co-localization of Ngb and the astrocytic marker-GFAP in human brain following acute ischemic stroke [23]. To the best of our knowledge, there has not been any studies either on Ngb expression of human astrocytes after TBI, or the astrocytic Ngb expression in chronic damaged brain of any species.

In western countries, TBI is responsible for the most fatalities under 45 years old and the World Health Organization has estimated that by 2020 TBI will be the most cause of disability worldwide. Unlike ischemic brain injury, TBI causes acute and primary morphological damage to brain tissues, leading to distinct pathological alterations. Hypoxia occurs secondarily to TBI, giving rise to enhanced expression of abundant hypoxia-inducible genes, such as hypoxia-inducible factor-1 alpha (HIF-1α), erythropoietin and vascular endothelial growth factor (VEGF). Accompanied by these genes, Ngb has also been demonstrated to have an enhanced expression to assist neuron-protection. However, its expression and role in glia cells following hypoxic/ischemic damage have not been stressed all the time. Therefore, in this study, we aimed to explore the expression of Ngb in glia under acute, sub-acute and chronic traumatic insults, respectively.

2. Materials and methods

Post-mortem brain specimens were obtained from West China Forensic Medicine Appraisal Center at Sichuan University and informed consent was obtained from the family members of the deceased. Tissues were collected in a short period of time after death from the Han ethnic group in Sichuan, including 15 objects who died of TBI and 3 control brain samples without neuropathy. Tissues were fixed with 4% paraformaldehyde (wt/vol), and then paraffin embedded. Detail information about the gender, duration and location of TBI was shown in Table 1. The following primary antibodies were used: mouse anti-GFAP (1:800, Sigma); mouse anti-NeuN (1:800, Millipore); rabbit anti-Olig2 (1:1000, Millipore); rabbit anti-Neuroglobin (1:100, BIOS, Beijing, China); mouse anti-Neuroglobin (1:100, Abcam). All secondary antibodies were donkey raised and diluted at 1:1000.

Embedded tissues were serial sectioned at 4 μm, and then hematoxylin and eosin (H&E) stains were processed for histological analysis. For immuno-staining, slices were permeabilized with 0.3% Triton X-100 in PBS for 10 min. 5% FBS blocking buffer incubated for at least 1 h to reduce nonspecific staining. Primary antibody was diluted in 5% FBS overnight at 4 °C, washed in PBS, followed by incubating with secondary antibodies for 2 h. For immunofluorescence, slides were blotted dry and mounted; for immunohistochemistry, DAB kit (ZSGB) was used as detection system.

Immunofluorescent images were captured by confocal laser scanning microscope (OLYMPUS FV1000 confocal imaging system). Alexa 488 were excited with a 488 nm Argon laser, Alexa 568 with a 546 nm HeNe laser, and Alexa 647 with the 633 nm HeNe laser. Light microscopy (Nikon 80i) was used to obtain H&E and immunohistochemistry images.

Table 1
Detail information of the human specimens.

TBI group				
Group	Survival periodafter TBI	Age (year)	Gender	Site of TBI
Acute	2 days	26	Male	Frontal lobe
	2 days	38	Female	Temporal lobe
	3 days	37	Female	Frontal lobe
Sub-	25 days	31	Male	Frontal, temporal lobe
acute	27 days	55	Male	Frontal, temporal lobe
	39 days	41	Female	Temporal lobe
Chronic	84 days	56	Male	Frontal,temporal lobe
3	92 days	26	Male	Frontal lobe
months	98 days	38	Female	Frontal lobe
Chronic	162 days	64	Female	Temporal lobe
6	174 days	56	Male	Frontal, temporal lobe
months	195 days	49	Male	frontal lobe
Chronic	367 days	27	Male	Frontal, temporal lobe
12	371 days	31	Female	Frontal lobe
months	381 days	73	Male	Frontal lobe

Control group		
Age (year)	Gender	Cause of death
32	Female	Amniotic fluid embolism
24	Male	Anaphylactic shock
65	Male	Arrhythmia

3. Results

3.1. Ngb was expressed in human astrocytes after TBI

We first focused our work on the chronic TBI group, which has been less stressed in the previous study of Ngb. To investigate the pathological alteration under chronic traumatic insult, we conducted H&E staining to chronic brain trauma specimens, and also normal human brains to serve as controls. Consistent with the general view, cytoplasm of glia cells was barely observed in normal human brains (Fig. 1A). The chronic traumatic brains exhibited abundant cytoplasm-enriched astrocytes and “fried-egg” like oligodendrocytes in the injury repairing center (Fig. 1B), indicating an activated state of these two types of glia. Immunohistochemical staining against Ngb was then performed in the two groups above to determine its expression pattern. In the control group, we observed mild expression of Ngb in cells with neuronal appearance (Fig. 1C). However, the TBI group displayed abundant Ngb immunoreactive cells that were morphologically resembling hypertrophic reactive astrocytes (Fig. 1D). The reactive astrocytes in the injured area, detected by immuno staining against GFAP (an astrocyte marker), were of large or middle size and sustained rich cytoplasm (Fig. 1F), compared with the fibrillary appearance of normal astrocytes (Fig. 1E).

To confirm the cell type of these Ngb-positive cells under chronic traumatic damage, we co-stained Ngb with markers for distinct neurocyte types. In chronic TBI, although most NeuN (differentiated neuron marker) immuno-reactive cells mildly exhibited