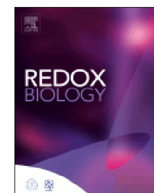
Redox Biology 18 (2018) 158–172

Contents lists available at ScienceDirect

Redox Biology

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



Research Paper

Breast cancer susceptibility protein 1 (BRCA1) rescues neurons from cerebral ischemia/reperfusion injury through NRF2-mediated antioxidant pathway

Pengfei Xu[a,1], Qian Liu[a,1], Yi Xie[a], Xiaolei Shi[b,c], Yunzi Li[a], Mengna Peng[a], Hongquan Quo[d], Rui Sun[e], Juanji Li[a], Ye Hong[a], Xinfeng Liu[a,*], Gelin Xu[a,*]

[a] Department of Neurology, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu 210002, China
[b] Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, British Columbia, Canada
[c] Department of Neurology, The First Affiliated Hospital, Yijishan Hospital of Wannan Medical College, Wuhu, Anhui 241001, China
[d] Department of Neurology, Jinling Hospital, Southern Medical University, Nanjing, Jiangsu 210002, China
[e] Department of Neurology, Jinling Clinical College of Nanjing Medical University, Nanjing, Jiangsu 210002, China

ARTICLE INFO

Keywords:
BRCA1
Ischemia/Reperfusion
Oxidative stress
DNA damage
NRF2

ABSTRACT

Cellular oxidative stress plays a vital role in the pathological process of neural damage in cerebral ischemia/reperfusion (I/R). The breast cancer susceptibility protein 1 (BRCA1), a tumor suppressor, can modulate cellular antioxidant response and DNA repair. Yet the role of BRCA1 in cerebral I/R injury has not been explored. In this study, we observed that BRCA1 was mainly expressed in neurons and was up-regulated in response to I/R insult. Overexpression of BRCA1 attenuated reactive oxygen species production and lipid peroxidation. Enhanced BRCA1 expression promoted DNA double strand break repair through non-homologous end joining pathway. These effects consequently led to neuronal cell survival and neurological recovery. Mechanically, BRCA1 can interact with the nuclear factor (erythroid-derived 2)-like 2 (NRF2) through BRCA1 C-terminal (BRCT) domain. The cross-talk between BRCT and NRF2 activated the NRF2/Antioxidant Response Element signaling pathway and thus protected injured neurons during cerebral I/R. In conclusion, enhanced BRCA1 after cerebral I/R injury may attenuate or prevent neural damage from I/R via NRF2-mediated antioxidant pathway. The finding may provide a potential therapeutic target against ischemic stroke.

1. Introduction

Ischemic stroke, with a sudden reduction of cerebral flow, induces neuronal damage and neurological dysfunction, which may further deteriorate after recanalization treatment [1,2]. Reactive oxygen species (ROS)-related oxidative damage has been recognized as the vital pathogenesis of neuronal loss and subsequent memory impairment following brain ischemia/reperfusion (I/R) injury [3–5]. Redox disruption has been associated with deteriorating clinical outcomes in acute ischemic stroke patients [6,7], but the underlying mechanisms remain largely unclear. The uncertainty in the mechanisms involved in cerebral I/R oxidative injury may be responsible for the ineffectiveness of current neuroprotective treatments. Therefore, a better understanding of pathological processes in I/R oxidative attack may provide a clue for establishing therapeutic targets of ischemic stroke.

Breast cancer susceptibility protein 1 (BRCA1), commonly known as

Abbreviations: ARE, antioxidative response element; AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; BRCA1, breast cancer susceptibility protein 1; BRCT, BRCA1 C-terminal; BER, base excision repair; CaMKII, calcium/calmodulin-dependent protein kinase II; CNS, central nervous system; CA1, cornu ammonis 1; DSBs, double strand breaks; DCX, doublecortin; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GPX3, glutathione peroxidase 3; GPX4, glutathione peroxidase 4; GCLC, glutamate-cysteine ligase catalytic; GCLM, glutamate-cysteine ligase regulator subunit; HO-1, heme oxygenase 1; HR, homologous recombination; 4-HNE, 4-hydroxynonenol; I/R, ischemia/reperfusion; Iba-1, ionized calcium-binding adapter molecule-1; MCAO, middle cerebral artery occlusion; NRF2, nuclear factor (erythroid-derived 2)-like 2; NQO1, NAD(P)H dehydrogenase (quinone 1); NHEJ, non-homologous end joining; NER, nucleotide excision repair; NPCs, neural precursor cells; NSCs, neural stem cells; 3-NT, 3-nitrotyrosine; 8-OHDG, 8-hydroxy-2′-deoxyguanosine; OGD, oxygen-glucose deprivation; PSD95, postsynaptic density protein 95; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; XRE, xenobiotic responsive element

* Correspondence to: Department of Neurology, Jinling Hospital, Medical School of Nanjing University, 305 East Zhongshan Road, Nanjing, Jiangsu 210002, China
E-mail addresses: xfliu2@vip.163.com (X. Liu), gelinxu@nju.edu.cn (G. Xu).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.redox.2018.06.012
Received 2 June 2018; Received in revised form 27 June 2018; Accepted 30 June 2018
Available online 07 July 2018
2213-2317/ © 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

a tumor suppressor, is encoded by the breast and ovarian cancer type 1 susceptibility gene [8]. BRCA1 exerts a crucial role in regulating oxidative stress [9]. BRCA1 can modulate intracellular ROS level by interacting with nuclear factor (erythroid-derived 2)-like 2 (NRF2) and promoting gene expression of antioxidant response element (ARE), such as HO-1, NQO1, GPX3 and SOD1 [10,11]. Moreover, BRCA1 can repair ROS-induced DNA double strand breaks (DSBs) [12]. In the central nervous system (CNS), BRCA1 is localized in hippocampus neurons [13]. BRCA1 deficiency has been coincided with increased genomic DNA damage, reduced neurite outgrowth and accelerated cognitive decline [14]. Interestingly, BRCA1 expression has been found to be elevated after I/R injury [15], indicating a potential compensatory effect of BRCA1 in I/R-related oxidative stress. Nevertheless, the role of endogenous BRCA1 in acute brain damage and long-term neurofunctional recovery following cerebral I/R has not been fully elucidated. To this end, in mice with middle cerebral artery occlusion (MCAO), we investigated the cellular expression and distribution pattern of BRCA1, explored the role of BRCA1 in neuronal oxidative stress, and addressed the functional domain of the protein directing the signaling transduction in neuroprotection.

2. Materials and methods

2.1. Lentiviral construction and orthotopic injection

Vector construction was performed by Cyagen Bioscience Inc (Guangzhou, China). To obtain BRCA1-encoding vector (pLV[Exp]-EGFP: T2A: Puro-EF1A-mBrca1), the entire musculus BRCA1 sequence was cloned in pLV/EF1A/EGFP plasmid. The vector pLV[Exp]-EGFP: T2A: Puro-Null was used as control. The virus was packaged using Lenti-XTM HTX packaging system (Clontech, USA). Briefly, HEK-293FT cells were transfected with BRCA1-encoding or control plasmid to produce lentivirus. Lentiviruses were further purified by CsCl gradient centrifugation (30,000 rpm, 4 °C, 16 h) to increase concentration (10^9 TU/mL). The purified lentiviruses were injected into the right lateral ventricle (dosage: 5 µl; coordinates: a/p, +0.4, m/l, +1.0, d/v, –3.5) and hippocampus (dosage: 3 µl; coordinates: a/p, −1.5, m/l, +1.0, d/v, –2.0) of mice using stereotaxic apparatus. The transfection efficiency was measured with GFP immunofluorescence and western blotting. MCAO surgery was performed 14 days after transfection.

2.2. Focal cerebral ischemia

Adult male C57BL/6 J mice (n = 171, weighting 20–25 g) were purchased from Model Animal Research Institute of the Nanjing University (Nanjing, China) and housed under controlled conditions (humidity 55–60%; temperature 23 ± 2 °C and 12-h-light-dark cycle) with food and water freely available. All experiments were approved by Experimental Animal Ethic Committee of Jinling Hospital and were implemented according to National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23, revised in 1996). Mice were randomized into four groups and received treatments as shown in Fig. S1.

Transient focal cerebral ischemia was induced by intraluminal MCAO surgery following previous method [16]. Briefly, after anesthetized with 2% isoflurane in O_2, the right common carotid artery, internal carotid artery (ICA) and external carotid artery (ECA) were carefully separated. Then a silicon-coated monofilament (diameter 0.16 mm) was inserted into ECA and advanced into ICA until mild resistance was felt. A decline of regional cerebral blood flow (rCBF) > 75% was considered successful interrupt of MCA blood flow monitored by a laser doppler flowmetry (PeriFlux 5010; Perimed AB, Sweden). After 90 min of occlusion, the monofilament was withdrawn for reperfusion. A heating pad was used to maintain the body temperature during surgery. The sham-operated mice underwent the same procedures except that the MCA was not occluded after the neck incision.

2.3. Cerebral infarct volume and brain edema determination

The 2, 3, 5-triphenyltetrazoliumchloride (TTC, Sigma, USA) staining was performed 24 h after reperfusion to measure infarct volume. The 1-mm-thick brain slices were stained with 2% TTC solution at 37 °C for 15 min in dark, followed by overnight fixing with 4% paraformaldehyde (PFA) at 4 °C. The stained slices were scanned using HP Scanjet G3110, and the infarct volume and edema ratio were analyzed with Image-Pro Plus 6.0 software (Media Cybernetics, USA) as reported by others [16]. Briefly, the relative infarct volume (%) was calculated as: (contralateral hemisphere volume − undamaged volume in ipsilateral hemisphere)/(contralateral hemisphere volume × 2)*100%, the relative edema volume (%) was calculated as: (ipsilateral hemisphere volume − contralateral hemisphere volume)/(contralateral hemisphere volume × 2)*100%.

2.4. Neurological deficit evaluation and behavioral analysis

The modified neurologic severity score (mNSS) was used to assess neurological deficits 24 h after reperfusion as described by Chen et al. [17]. The scoring system contains three tests including motor test, sensory test and beam balance test, in which 0 represents no deficit and 14 represents maximal deficits.

Open field test (OFT) was performed to evaluate spontaneous activity and adaptability for 10 mice of each group at day 21 after MCAO surgery using a computerized tracking system (Noldus EthoVision XT, shanghai, China). The animals were placed individually in the corner of an open square (50 cm × 50 cm × 40 cm) in a quiet environment, the total distance traveled, the rearing activities, the time spent and the frequency of entry into the central area ($30 \times 30\ cm^2$) were automatically recorded by a digital camera on the top of the square for 30 min per mouse.

Spatial learning and memory were evaluated at day 22–28 after surgery by Morris Water Maze (MWM) test [18]. Firstly, the mice were trained to find the escape platform with cues. Then the mice were trained to find the platform in four trials per day for 5 consecutive days (n = 10 mice per group). If mice failed to reach the platform within 60 s, they would be manually guided to the platform and remained there for 10 s before being placed back to cage. At last, the platform was removed to perform probe trial, and each subject was allowed to search the platform for 60 s. The escape latency to find the platform, the swim path length, the time spent in the target quadrant and platform crossovers were tracked and analyzed by the ANY-maze video tracking software (Stoelting, USA).

2.5. Histological staining and MDA assay

Mice were deeply anesthetized with chloralhydrate (120 mg/kg, i.p.) and intracardially perfused with phosphate-buffered saline (PBS) followed by 4% PFA. Brains were then embedded in optimal cutting temperature compound (Sakura Finetek, USA) and cut coronally into 15 µm or 25 µm sections. Brain sections or neuronal coverslips were fixed with 4% PFA for 20 min before immunostaining. The levels of MDA were detected by Lipid Peroxidation MDA Assay Kit (Beyotime, China).

2.6. Immunofluorescence and TUNEL staining

Sections were blocked in a solution containing 5% goat serum, 1% BSA and 0.3% Triton X-100 for 1 h. The slices or coverslips were incubated with primary antibodies overnight against BRCA1 (1:200, Abcam, UK), NeuN (1:500, Millipore, USA), Iba1 (1:100, Santa Cruz Biotechnology, USA), GFAP (1:500, Abcam, UK), γH2A.X (1:200, Millipore, USA), GFP (1:200, Cell Signaling Technology, USA), 8-OHDG (1:200, Abacm, UK), DCX (1:200, Abcam, UK) and MAP2 (1:200, Cell Signaling Technology, USA). Then the specimens were incubated with

Download English Version:

https://daneshyari.com/en/article/8286330

Download Persian Version:

https://daneshyari.com/article/8286330

Daneshyari.com