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Research report

Suppression of FoxO3a attenuates neurobehavioral deficits after traumatic brain injury through inhibiting neuronal autophagy

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ABSTRACT

Traumatic brain injury (TBI) is a serious insult that frequently leads to neurological impairments. Forkhead box O (FoxO) 3a, as transcription factor, has been confirmed to modulate autophagic process. Moreover, FoxO3a is expressed throughout the brain including the hippocampus. However, the role of FoxO3a in the pathophysiology of TBI is unclear. The present study is designed to investigate whether FoxO3a has the neuroprotective effects on rats subjected to TBI, and further to explore the potential molecular mechanisms. Thus, a rat model of TBI was created by using a modified weight-drop device to mimic the insults of TBI. The results showed that FoxO3a was significantly increased in the serum of patients with TBI as well as in experimental animals. Furthermore, our data also demonstrated that TBI stimulated the translocation of FoxO3a from the cytosol to the nucleus. Additionally, we found that knockdown of FoxO3a by siRNA silencing significantly improved neurobehavioral dysfunctions and conferred a better neuroprotective effects after TBI, evidenced by promoting motor behavioral recovery, attenuating learning and memory impairments, and partially reversing neuronal damage in the hippocampus. To further investigate the molecular mechanisms underlying this neuroprotection, we identified that nuclear accumulation of Foxo3a could induce highly expression of autophagy pathway genes including LC-3, Beclin-1, p62, ATG12, and ATG14, and finally initiate neurological impairments. Interestingly, silencing FoxO3a by siRNA remarkably inhibited the induction of neuronal autophagy after TBI, and activated autophagy was closely related to TBI-induced neurological deficits. Taken together, these findings indicated that FoxO3a knockdown conferred neuroprotective effects after TBI through inhibiting the activation of neuronal autophagy.

1. Introduction

Traumatic Brain Injury (TBI) is one of the most important causes of death and long-term neurological impairments, especially in children and young adults [1]. TBI induces delayed progressive tissue damage through a series of bio-physiological and pathological reactions [2]. These lead to subsequent neuronal cell death and neurological dysfunctions, such as motor, sensory, learning and memory impairments [3]. Strikingly, increased markers of autophagy have been reported in the rat hippocampal neurons following TBI in our previous study [4]. Additionally, We have recently found that activated autophagy participated in the pathophysiological process of TBI [5]. Furthermore, the activation of neuronal autophagy might be associated with TBI-induced neurological impairments. However, the upstream molecular mechanisms of autophagic activation remains undetermined.

FoxO, one of evolutionarily conserved proteins, has been implicated in regulating diverse cellular functions, including proliferation, differentiation, metabolism, oxidative stress, and cell longevity as transcription factor [6]. Mammalian cells express some FoxO isoforms, such as FoxO1, FoxO3a and FoxO4 [7]. Among them, although FoxO1 and FoxO3a share overlapping structure and function, they have different tissue-dependent expression patterns. FoxO1 is highly expressed in adipose tissues, whereas FoxO3a is most highly expressed in brain tissue including the hippocampus, cerebral cortex and cerebellum [8]. Recent studies have revealed that FoxO3a is associated with brain damage after cerebral ischemia and has the potential to promote stroke, thus suppression of FoxO3a exerts neuroprotective effects against ischemic injury [9–12]. Furthermore, FoxO3a has been implied in neuronal apotheosis after hypoxic-ischemic brain injury in neonatal rats [9,10]. It has been reported that FoxO3a is activated and translocated

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from the cytosol to nucleus and mediates neuron death in response to β-amyloid toxicity in Alzheimer's disease (AD), which is correlated with preservation of spatial reference memory [13,14]. However, little is known about the potential effect of FoxO3a on neuronal autophagy-induced neurological deficits after TBI.

FOXO3a has been reported to be regulators of cellular autophagy, a process that degrades long-lived cellular proteins and damaged organelles as a critical cell survival mechanism in response to stress [15]. Activated FoxO3a stimulates autophagy through increasing the transcription of many autophagy-related genes (ATG) and regulator genes, such as microtubule-associated protein 1 light chain 3 (LC-3), Beclin-1, ATG5, ATG7, ATG12 and ATG14 [16,17]. Accumulating evidences have indicated that FOXO3a-mediated autophagy was observed in skeletal muscle [15], hematopoietic stem cells [18], cardiomyocytes [19] and cancer cells [20]. A new report has revealed that nuclear translocation of FoxO3a and various essential ATGs were increased in ethanol-treated primary hepatocytes, and acute ethanol-treated Foxo3a-/- mice exhibited decreased autophagic activity [17], Moreover, knockdown of FoxO3a might prevent globular adiponectin-induced expression of LC-3II and ATG5 in macrophages [21]. In fibroblasts derived from patients with idiopathic pulmonary fibrosis (IPF), Im J et al. has identified that FoxO3a binds to the promoter region of LC-3II and transcriptionally activates LC-3II by using the luciferase assay [22]. However, the role of FoxO3a in TBI is not well documented.

In the present study, we investigated the biological function and molecular mechanism of FoxO3a in TBI model as transcription factor. Firstly, we found FoxO3a was significantly elevated in TBI patients and experimental TBI rats, which was associated with degree of brain injury. Secondly, silencing FoxO3a by siRNA not only improved TBI-induced neurobehavioral deficits and neuronal injury in the hippocampus, but also suppressed TBI-induced neuronal autophagy involved in neurological deficits. Finally, the results suggested that FoxO3a knockdown conferred neuroprotective effects after TBI through inhibiting the activation of neuronal autophagy. This study might offer a novel therapeutic strategy for TBI by modulating FoxO3a levels.

2. Materials and methods

2.1. TBI patients and blood specimens

A total of 60 patients diagnosed with TBI according to clinical information and head computed tomography (CT) analysis were recruited in the Department of Neurosurgery, Beijing Tiantan Hospital of Capital Medical University (Beijing, China), from January to December 2015. The inclusion criteria: (1) an injury event (i.e., blast, fall, motor-vehicle crash, head impact) occurred a within the preceding 24 h; (2) loss of consciousness (if present) for < 24 h. (3) 13–15 Glasgow Coma Scale (GCS) considered mild TBI, 9–12 GCS scores considered moderate TBI (n = 16), and 3–8 GCS scores considered severe TBI (n = 16). Clinical characteristics of these patients were shown in Table 1. They have no accompanying disease conditions like chronic inflammation, arthritis, diabetes and cancer etc. The age-matched healthy subjects were gathered as control groups (n = 20). The study was approved by the Human Ethics Committee of Capital Medical University and written informed consent was obtained from all participants. Blood was collected from all subjects for assessments.

Table 1
The clinical characteristics of TBI patients.

Variable	Value
Age	33.3 ± 8.5
Sex	Male 38 Female 22
GCS (initial)	11.7 ± 4.2
Initial systolic blood pressure (mmHg)	130.4 ± 22.4
Injury Severity Score (ISS)	19.5 ± 5.3
Intracranial pressure monitoring (ICP)	25.7 ± 8.1
Unilateral dilated pupil	1
Midline shift (mm)	5.4 ± 1.2
Herniation	2
Acute respiratory faiure (ARF)	1

2.2. Animal and experimental design

A total of 120 male Sprague-Dawley (SD) rats (aged 12 weeks and weighing 350–375 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Vital River, Beijing, China). All rats were randomly divided into seven groups: Sham group, TBI group, TBI + siFoxO3a-1 group, TBI + siFoxO3a-2 group, TBI + siControl group, TBI + 3-MA group, TBI + CBX group. Animals were housed with a standard of 12 h light/dark cycle and free access to water and food before and after operation. All experimental procedures were carried out in accordance with the guidelines of the Chinese Council on Animal Protection, and approved by the Ethics Committee of Capital Medical University for the use of animals in this study. Enormous efforts have been made to minimize animal suffering in the operation.

2.3. RNA interference (RNAi) and transfection

For specific gene knockdown on FoxO3a mRNA, the lentiviral particles of siRNA targeting Foxo3a (siFoxo3a) and scramble siRNA Control (siControl) were purchased from Genechem Co. Ltd (Shanghai, China). The sequence of siRNA is as followed: siRNA-1 of FoxO3a (siFoxO3a-1, 5′-AUUGACCAAACUUCCCUGGUUAGGC-3′), siRNA-2 of FoxO3a (siFoxO3a-2,5′-GAGCUCUUGGUGGAUCAUC-3′) [36]. These lentiviral particles injected into mouse brain as previously described. Rats received a single right intracerebroventricular injection of lentiviral particles 5.0 μl (10^7–10^8 pfu/ml) [23]. The Hamilton brain infusion syringe (Hamilton, Nevada, USA) was stereotaxically injected into the right lateral ventricle (coordinates: 1.5 mm caudal to bregma; 1.1 mm lateral to midline; 4.5 mm deep from the surface of skull). The knockdown efficiency of FoxO3a was detected by real-time PCR and Western blot analysis.

2.4. TBI model

Seven days after lentiviral infection, the rat model of TBI was induced by using a modified weight-drop device, as described previously by Marmarou [24]. Additionally, autophagy inhibitor 3-methyladenine (3-MA) 1 μl (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was administered by right ventricle injection 30 min before TBI induction [25]. The survival rate of rats in each group as follows: total eight rats were died in the experiment, four rats were in the TBI group, two were in the TBI + siFoxO3a-1 group, one was in the TBI + siFoxO3a-2 group, and one was in the TBI + CBX group.

2.5. Accelerating rotarod test

Motor coordination and balance were assessed by using the accelerating rotarod test at 7 d post-TBI. This test was performed as previously described [26]. Briefly, Animals were trained for three days prior to TBI or sham injury, and placed on the rotarod, consisting of a rotating spindle 7 cm wide, and had to continuously walk forward to avoid falling. The rotarod started at an initial speed of 4 RPM and then accelerated to 40 RPM over the course of 300 s. 360 s was the maximum time allowed on the rotarod. The average latency to fall from the rotating rod during the testing period was calculated for each rat.

2.6. Modified neurological severity score test

Posttraumatic neurological impairments were further assessed using the modified neurological severity score (mNSS) test at 7 d post-TBI,