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Original Article

The mechanism of chemokine receptor ACKR2 in cognitive impairment in young rats with traumatic brain injury

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Abstract: Objective: To investigate the role of chemokine receptor ACKR2 in cognitive impairment in young rats with traumatic brain injury. Methods: Seventy-five young rats were randomly divided into TBI group, TBI+Negative control (NC) group, TBI+Sensory integration (SI) group, TBI+Atypical chemokine receptor 2 (ACKR2) group and TBI+SI+ACKR2 group. We employed several techniques, including the water maze test, transmission electron microscope, HE staining, enzyme-linked immunosorbent assay, RT-PCR, western blotting to elucidate the role of ACKR2 in young rats with traumatic brain injury. Results: We observed that the ability of learning and memory were significantly decreased, the serum inflammatory factors CCL2, IL-1β and TNF-α were increased, the expression of Tau and NG2 was increased, and the expression of ACKR2 was decreased in TBI rats. After ACKR2 overexpression combined with SI, the learning ability and memory ability of TBI rats were improved, the levels of CCL2, IL-1β, TNF-α were decreased, the expressions of Tau and NG2 were decreased, which were better than those of SI and ACKR2 groups. Conclusion: ACKR2 may be an effective method for improving the results after TBI in young rats.

Keywords: ACKR2, traumatic brain injury, cognitive impairment

Introduction

With the development of modern industry, power machinery and high-speed transportation, the incidence of traumatic brain injury (TBI) is constantly increasing [1]. Globally, traumatic brain injury is one of the leading causes of death and disability [2]. Compared with adults, children are more prone to craniocerebral injury because of their stronger athletic ability and weaker self-protection ability. After TBI, the degree of cognitive dysfunction is directly related to the efficacy of rehabilitation training in children, which directly affects patients' motor function, daily living ability and social adaptability. Cognitive dysfunction brings many difficulties for patients to return to society and becomes a major problem in rehabilitation treatment. It is still unclear how cognitive dysfunction occurs, however. Yet no pharmacological intervention has been shown to be efficacious in improving patient outcomes [3]. There is therefore a need to explore alternative therapeutic targets in the brain, which could aid in the development of future pharmacological interventions. One such alternative may lie in the modulation of the brain's inflammatory events induced by injury [4, 5].

The theory of sensory integration (SI) was first systematically proposed by Dr. Ayers, an American clinical psychologist, based on the study of brain function, occupational therapy and experimental results. It refers to the ability of the body to use its own senses to input information obtained from different sensory pathways from the environment to the brain, and the brain processes the input information and

makes adaptive responses [6]. Since the 1970s, SI approach or sensory integrative therapy has been used for the treatment of challenged children. Several studies have demonstrated the effectiveness of the SI approach for children with cognitive impairments [7]. Sensory integration training can correct and improve the behavior, cognitive ability and social communication ability, etc. and make them as close to the level of normal children as possible.

In contrast to classical chemokine receptors, atypical chemokine receptor 2 (ACKR2, previously known as D6) does not sustain directional cell migration after ligand engagement [8]. Instead, because of its constitutive recycling from the plasma membrane vesicles and back to the plasma membrane [9], it internalizes proinflammatory CC chemokines, providing support for their degradation and clearance from the extracellular space [10]. In vivo, this scavenger function has been confirmed. Healthy and fertile ACKR2-/- mice display no overt differences in phenotype [11], while models of skin inflammation [12] and experimental colitis consistently [13] show an exacerbated inflammatory response. Of note, in all experimental settings, inflammatory chemokines were found more concentrated in inflamed tissues and draining lymph nodes.

A variety of tissues express ACKR2, including lymphatic endothelial cells of the lung and skin, as well as syncytiotrophoblasts in the placenta [14]. It has been reported that ACKR2 is expressed in the central nervous system [15], but no details are known about its anatomical distribution or regulation under pathological conditions, nor its biological functions. Considering the scavenging function of ACKR2 in inflammatory CC chemokines and its known roles in TBI, we hypothesized that the absence of ACKR2 could lead to an increase in brain level of inflammatory CC chemokine CCL2, which causes leukocyte infiltration, possibly by modifying neurological function recovery after brain injury.

Materials and methods

Laboratory animals

Sixty SPF 18-day-old Sprague-Dawley (SD) rats (Beijing Vital River Laboratory Animal Technology Co., Ltd., Certificate number: SCXK (Jing) 2018-0025) were raised at a constant temperature of (24±1°C) with one in each cage, followed by free diet and drinking water in dark/light cycle of 12 hours, and the experiment was started after one week of adaptive feeding. The study was approved by The First Affiliated Hospital of Nanchang University.

Reagents

TRIzol (TaKaRa, Otsu, Shiga, Japan); PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan); TaqMan® miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA); SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan); Chemokine ligand 2 (CCL2) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, 9054-89-1); Interleukin-1 beta (IL-1β) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, R-KMLJr34440); Tumor necrosis factor-alpha (TNF-α) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China, R-KMLJr34981); Microtubule-associated protein tau (Tau), NG2, Atypical chemokine receptor 2 (ACKR2), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Proteintech, Wuhan, Hubei, China); ACKR2 overexpression adenovirus vector (OBiO Technology, Shanghai, China).

The TBI experimental model

Brain injury model was established using animal craniocerebral injury device [16]. 60 rats were anesthetized by inhalation of ether, the rat skull was fixed on the device by using the head clamp, the incisor hole and the two ear sticks, and the torso was at a 90°C to the horizontal. Press the trigger, the rat cranial was rotated laterally, and the rat TBI model was built. After injury, the rats were immediately put into an incubator to observe the changes of vital signs after injury. When the signs were stable, the rats were put into the animal cage, and Morris water maze test were used to test the changes of learning and memory function of rats to confirm the model was successful.

Model rats were randomly divided into traumatic brain injury (TBI) group, TBI+Negative control (NC) group, TBI+Sensory integration (SI) group, TBI+ACKR2 (ACKR2) group, and TBI+SI+ACKR2 overexpression (SI+ACKR2) group. The SI group received sensory integration training based on conventional rehabilitation thera-

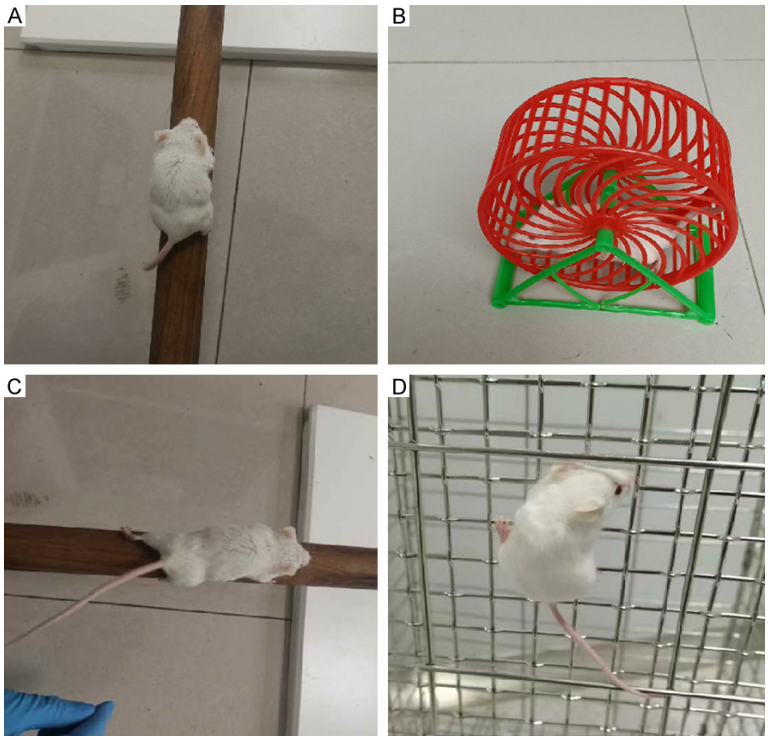

Figure 1. Sensory integration training. A. Balance beam training. B. Rotation training. C. Rotary rod training. D. Grille training.

py every day. The ACKR2 group was injected 5 × 10^7 TU ACKR2 lentiviral vector with a titer of (2 × 10^9 TU/mL) through lateral ventricle localization, once a week, the SI+ACKR2 group was injected 5 × 10^7 TU ACKR2 lentiviral vector with a titer of (2 × 10^9 TU/mL) through lateral ventricle localization once a week in addition to sensory integration training every day at the same time. The NC group was injected 5 × 10^7 TU empty lentiviral vector with a titer of (2 × 10^9 TU/mL) and the TBI group was injected with physiological saline through lateral ventricle localization, once a week. After stopping the needle for 1 min, the needle was slowly withdrawn and closed with bone wax, continued for 8 weeks. 10 normal rats were used as control group.

Sensory integration training [17]

Sensory integration training on grasp, rotation, walking and balance was conducted for 30 minutes per day and continuous for a week, as shown in **Figure 1**.

A: Balance beam training: The rats were made to walk on a square stick (100 cm long and 2.5 cm wide), which was placed on a container of water.

B: Rotation training: The rats were placed into a circular mesh instrument (6.5 cm in length and 12.5 cm in diameter), then rotated at 5 r/min for training. The purpose of this instrument is for the training of grasping, rotation and walking.

C: Rotary rod training: A pole with a length of 150 cm and a diameter of 4.5 cm was used for the training. The rats were placed on one end of the pole, then the pole was rotated alternately left and right at a speed of 3 r/min. This program is for the training of grasping and balance.

D: Grille training: The grille size was 50 cm × 40 cm. A 2.5 cm high wood frame was placed on the left, right and top of grille, and the grille was placed at a height of 80 cm. The grille was placed horizontally, the rat was placed on it, and then slowly raise one end of grille and turn it into a vertical position within 2 s. Hold for 5 s and observe whether the rats fell off the grille or grasped the grille with its front paws.

Morris water maze

Morris water maze: A circular water tank with a diameter of 180 cm and a height of 60 cm, in which there were a circular transparent platform with a diameter of 20 cm and a data acquisition and analysis system, was applied for the test. (1) Spatial learning ability test: On the 4th day, the rats were given navigation training from 9:00 a.m. The rats were randomly placed at the four edges of the pool, and the time for the rats to find the circular transparent platform was recorded; those failed to find platform was recorded for 120 s. The test was done 4 times a day for 4 days. The escape latency was calculated by calculating the average navigation time of the rats to reach the platform every day, longer escape latency indicates poorer cognitive function. (2) Space exploration test: All the rats were tested for memory on the 7th day. The circular transparent platform was removed at 9 a.m., and the rats were randomly placed at the four edges of the pool, with a sail-

ing time of 60 s. The time of rats crossing the original platform was recorded. The test was performed twice with an interval of 15 min, and the average value was taken.

Brain tissue staining

The next day after intervention, the brain tissue was removed and prepared into routine paraffin sections. The paraffin sections were fixed in xylene for 30 min. They were then dehydrated in 100%, 95%, 85% and 75% ethanol solution for 5 min, respectively, Hematoxylin stained for 10 min, soaked in 1% hydrochloric acid for 5 s before washing. Then, the tissues were stained with eosin dye for 3 min, dehydrated 2 min, followed with transparent treatment with 75%, 85%, 95%, 100% ethanol, xylene, and then sealing with neutral resin. Five clear visual fields were selected for each section to observe the changes of brain tissue.

Transmission electron microscope

The hippocampus was dissected into 1 × 1 × 1 mm-sized sections, and immediately placed in 2.5% glutaraldehyde in 0.1 mol/L PBS at 4°C overnight. After three rinses in 0.1 mol/L PBS, the sections were immersed in 1% osmium tetroxide in 0.1 mol/L PBS for 2 hours at 4°C. The tissue block was dehydrated in graded ethanol solutions and embedded in epoxy resin. Polymerized samples were cut into 70-nm-thick sections using an LKB-I microtome after 48 h of polymerization at 60°C. After staining with 50% ethanol saturated uranyl acetate solution for 1 hour and lead citrate solution for 15 minutes at room temperature, the sections were observed under a TEM.

Enzyme-linked immunosorbent assay

Rats were fasted without water for 12 hours before sampling, and anesthetized with IP 10% chloral hydrate (3 mL/kg). Blood was then taken from the abdominal aorta, and centrifuged at 3500 r/min. The supernatant was collected to detect CCL2, IL-1β and TNF-α according to the kit instructions.

Immunohistochemical staining

Brain tissue was taken from each group of rats. The samples were embedded in paraffin, sliced at 4 μm, baked for 2 h, dewaxed twice with xylene, hydrated with gradient ethanol, and placed in boiling sodium citrate solution for antigen repair, and cooled naturally to room temperature. Then, the samples were washed with PBS for 3 times, and endogenous peroxidase blocker was dropped and incubated for 10 min at room temperature. Later, the samples were washed with PBS for 3 times, and non-immune animal serum was added and incubated for 10 min at room temperature. After serum was removed, rabbit polyclonal antibodies against CCL2, IL-1β and TNF-α (1:150, 1:150, 1:50, respectively) were added and incubated overnight at 4°C. The samples were rinsed with PBS for 3 times, and biotin-labeled sheep anti-rabbit IgG (secondary antibody) was added, incubated at room temperature for 10 min, rinsed with PBS for 3 times, and streptomyces antibiotin-peroxidase was added at room temperature for 10 min. The samples were rinsed with PBS for 3 times, followed by DAB for color development, rinsed with distilled water, and counterstained with hematoxylin. Finally, the samples were rinsed with running water, followed by gradient ethanol dehydration and drying, xylene transparent, neutral gum seal, and light microscope observation, with brown for positive expression. Image-pro Plus 6.0 image analysis software was used for semi-quantitative analysis. Five non-repeated high-power fields were randomly selected for each section to calculate the integrated absorbance of positive staining (IA) and the area of positive region (Area), and their average values were taken for analysis. Relative protein expression was expressed as mean absorbance (IA/Area).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

The hippocampus was quickly dissected on ice after blood was taken from the abdominal aorta. The total RNA, extracted from tissues was routinely extracted by TRIzol method. The genomic DNA was removed by deoxyribonuclease I. Reverse transcription was performed following the kit's instructions. Primers included in the qPCR primer panels are shown in **Table 1**. The fluorescence quantitative PCR reaction system was 25 μl, containing 500 ng cDNA template, 250 nmol/L upstream and downstream primers and 12.5 μl 2 × SYBR Green PCR Master mixture. The reaction conditions were as follows: 94°C for 40 s, 55°C for 40 s,

Table 1. Primer sequences

	Primer sequence (5'-3')	Amplified fragment (bp)
GAPDH	F 5'-CATCACCATCTTCCAGGAGCGAGA-3'	239 bp
	R 5'-TGCAGGAGGCATTGCTGATGATCT-3'	
Tau	F 5'-CCTGAGCAAAGTGACCTCCAAG-3'	98 bp
	R 5'-CAAGGAGCCAATCTTCGACTGG-3'	
NG2	F 5'-GTCCTCCTGCTCCTGGCTCTC-3'	173 bp
	R 5'-ACAGTTGTGAGTGGAATGGCTTGG-3'	
ACKR2	F 5'-GACTACGCACTCCAGGTAACAG-3'	143 bp
	R 5'-AAGCCTTCAGGTACTGGCGGAA-3'	

72°C for 40 s, with a total of 45 cycles. The relative expression of the gene was represented by the value of $2^{-\Delta\Delta Ct}$, and the Ct value represented the number of amplification cycles in the process of PCR amplification when the fluorescence signal of the amplified product reached the set threshold, ΔCt = sample to be tested (Ct target gene - Ct GAPDH) - control group (Ct target - Ct GADPH). P<0.05 was considered statistically significant.

Western blot analysis

The hippocampus tissue was lysed by RIPA (Beyotime). 30 min later, the supernatant was collected for centrifugation, and the total tissue protein was extracted. The protein concentration was determined by BCA method. 20 μL protein samples were sampled. After electrophoresis, the protein was transferred to 200 mA membrane by electroporation, followed by washing membrane with 5% skim milk. PVDF membrane was closed at room temperature for 1 h, and then diluted Tau (1:500), NG2 (1:500), ACKR2 (1:250) and internal reference β-actin (1:250) antibodies were added and incubated overnight in a shaker at 4°C, followed by adding second antibody. Following a wash with TBST for 2 h at room temperature and ECL staining, the protein expression was observed in a gel imager, and the image analysis was performed with Quantity one software.

Statistical analysis

Analysis of the data was carried out using the statistical software SPSS22.0. Data conforming to normal distribution were expressed as mean ± standard deviation, and the comparison between two groups was conducted using the dependent sample t test. Comparison among groups was performed using single factor analysis of variance, and LSD-t method was performed for post-hoc pairwise comparison. P<0.05 was taken for a statistically significant difference.

Results

Effects of ACKR2 overexpression on cognitive behavior in TBI rats by water maze test

Compared with the control group, the escape latency was significantly prolonged (P<0.01), and the frequency of crossing the platform was significantly decreased in the TBI group (P<0.01). Compared with TBI group, the escape latency was significantly shortened (P<0.05, P<0.01), and the frequency of crossing the platform was significantly increased in the SI group, ACKR2 group and SI+ACKR2 group (P<0.05, P<0.01), as shown in **Figure 2A-C**.

Effects of ACKR2 overexpression on histopathology of brain tissues in TBI rats by HE staining

HE staining showed that in the control group, cerebral cortex was normal in morphology and structure, there were a large number of nerve cells with abundant cytoplasm, and the nucleus was large and round (**Figure 3A**). Compared with control group, there were serious edema in nerve cells, condensed nuclei, nuclear loss in the TBI and TBI+NC group (**Figure 3B**, **3C**). In the SI, ACKR2, and SI+ACKR2 group, the brain tissues were less edematous, and the neurons possessed clearer nuclei (**Figure 3D-F**).

Effects of ACKR2 overexpression on ultrastructure of neurons and synapses by transmission electron microscopy

The ultrastructure of neurons in control group was normal (**Figure 4A**). In the TBI and TBI+NC models, brain injury seriously disrupted the integrity of neurons, characterized by translucent cytoplasm, swelling mitochondria and breakdown of synaptic structure (**Figure 4B**, **4C**). The neurons' integrity in ACKR2 and SI+ACKR2 group improved obviously compared with TBI group (**Figure 4D-F**).

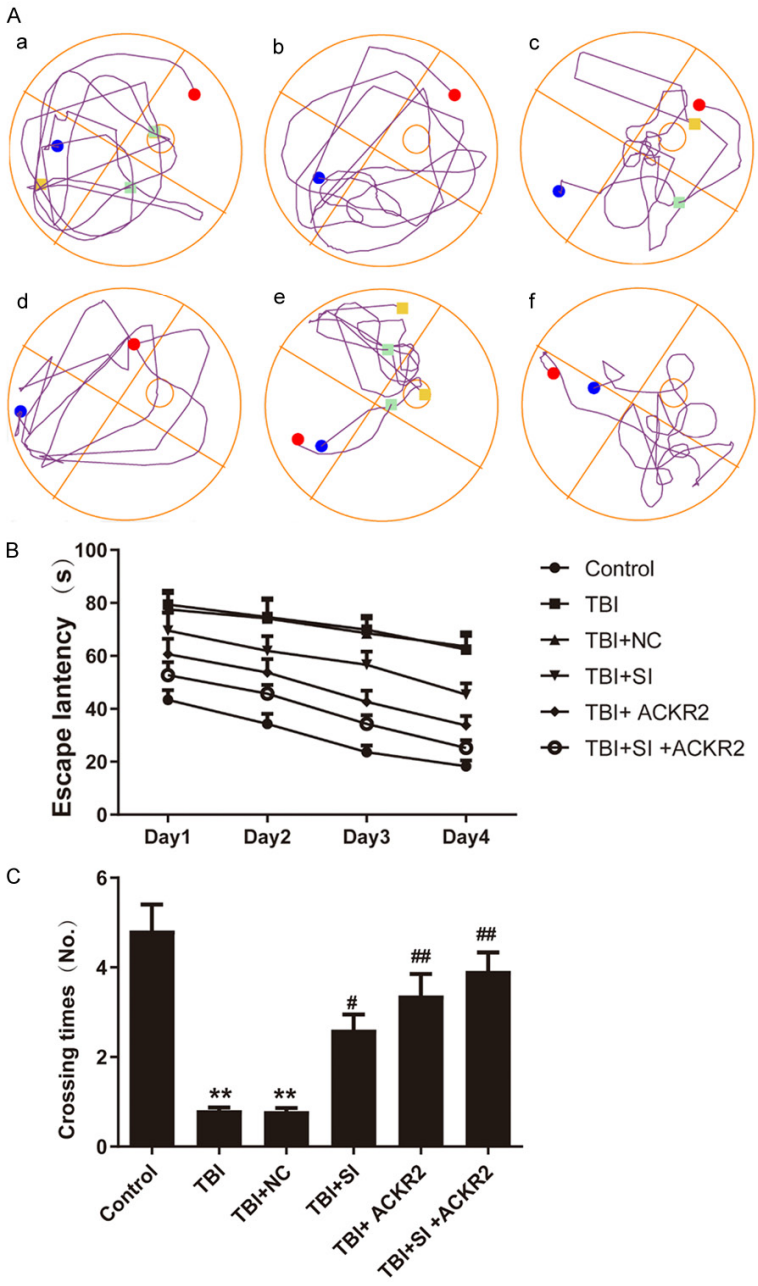

Figure 2. Effects of ACKR2 overexpression on cognitive behavior in TBI rats by Water maze test ($\bar{x}$ ± sd, n = 10). Note: *P<0.05, **P<0.01, compared with the Control group; #P<0.05, ##P<0.01, compared with the TBI group. A. Representative traces of swimming plot in Morris water maze test. B, C. The quantitative analyses of the Morris water maze. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI).

Effects of ACKR2 overexpression on CCL2, IL-1β, TNF-α levels in TBI rats by immunohistochemical staining

Compared with control group, CCL2, IL-1β, TNF-α levels were significantly increased in TBI group and TBI+NC group (P<0.01). Compared with TBI group, CCL2, IL-1β, and TNF-α levels were significantly decreased in SI group, ACKR2 group and SI+ACKR2 group (P<0.05, P<0.01), as shown in **Figure 5A-C**.

Effects of ACKR2 overexpression on Tau, NG2, ACKR2 mRNA expression in TBI rats by RT-qPCR

Compared with control group, the mRNA expression of Tau and NG2 was significantly increased and the mRNA expression of ACKR2 was significantly decreased in TBI group and TBI+NC group (P<0.01); Compared with TBI group, the mRNA expression of Tau and NG2 was significantly decreased and the mRNA expression of ACKR2 was significantly increased in SI group, ACKR2 group and SI+ACKR2 group (P<0.05, P<0.01), as shown in **Figure 6A-C**.

Effects of ACKR2 overexpression on Tau, NG2, ACKR2 protein expression in TBI rats by western blot

Compared with control group, the protein expression of Tau and NG2 was significantly increased and the protein expression of ACKR2 was significantly decreased in TBI group and TBI+NC (P<0.01). Compared with TBI group, the protein expression of Tau and NG2 was significantly decreased and the protein expression of ACKR2 was significantly increased in *ACKR2 group* and SI+ACKR2 group (P<0.01), as shown in **Figure 7A-D**.

Discussion

After traumatic brain injury (TBI), the inflammatory response plays an important role in the

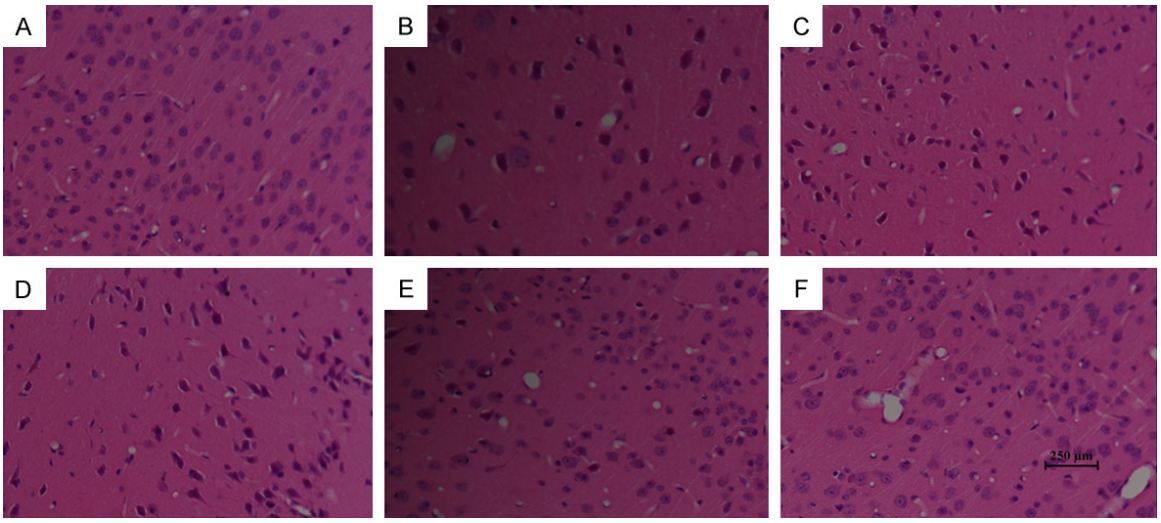

Figure 3. Effects of ACKR2 overexpression on histopathology of brain tissues in TBI represented by HE staining (× 200). (A) Control group: Cerebral cortex was normal in morphology and structure, there were a large number of nerve cells with abundant cytoplasm, the nucleus was large and round. (B) TBI group and (C) TBI+NC group: Serious edema in nerve cells, condensed nuclei, nuclear loss. (D) TBI+SI group, (E) TBI+ACKR2 group, (F) TBI+SI+ACKR2 group: The brain tissues were less edematous and the neurons possessed clearer nuclei. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI).

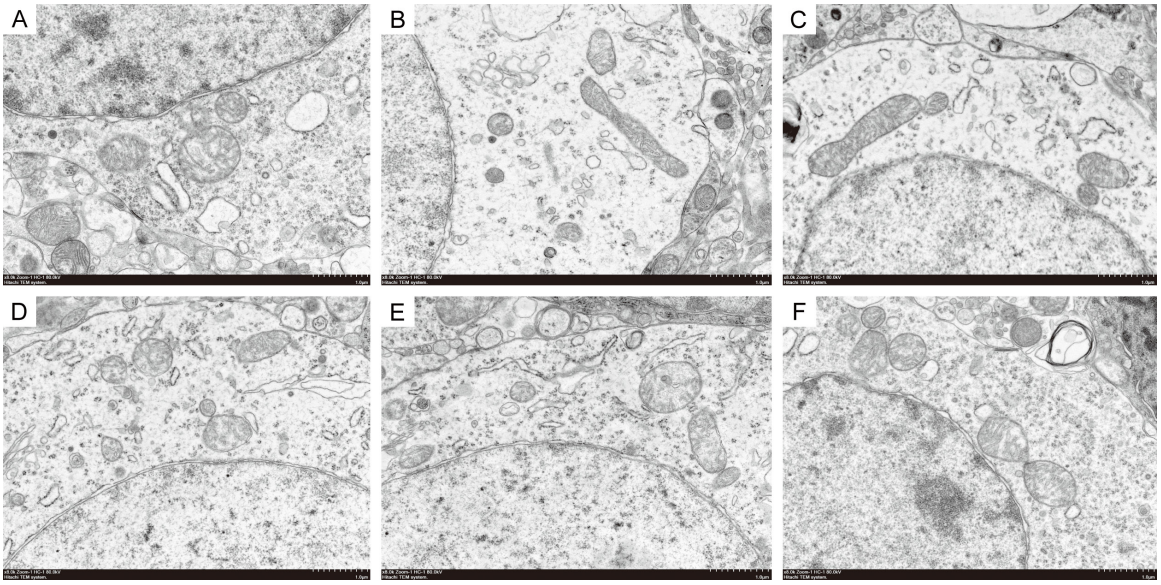

Figure 4. Effects of ACKR2 overexpression on ultrastructure of neurons by transmission electron microscopy. (A) Control group: The neuronal cell structure was normal and the organelles were intact. (B) TBI group and (C) TBI+NC group: Neuronal cells showed obvious edema, reduced organelles and vacuolated mitochondria. (D) TBI+SI group: Neuronal cell edema was reduced, mitochondria were vacuolated. (E) TBI+ACKR2 group: Slightly dilated endoplasmic reticulum with Golgi apparatus. (F) TBI+SI+ACKR2 group: Close to normal neuronal structure. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI).

secondary brain injury. Inflammation can continuously amplify the immune abnormality, thus affecting the stability of the blood-brain barrier [17], and the impairment of the blood brain barrier (BBB) can lead to vasogenic brain edema, causing abnormally elevated levels of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) [18].

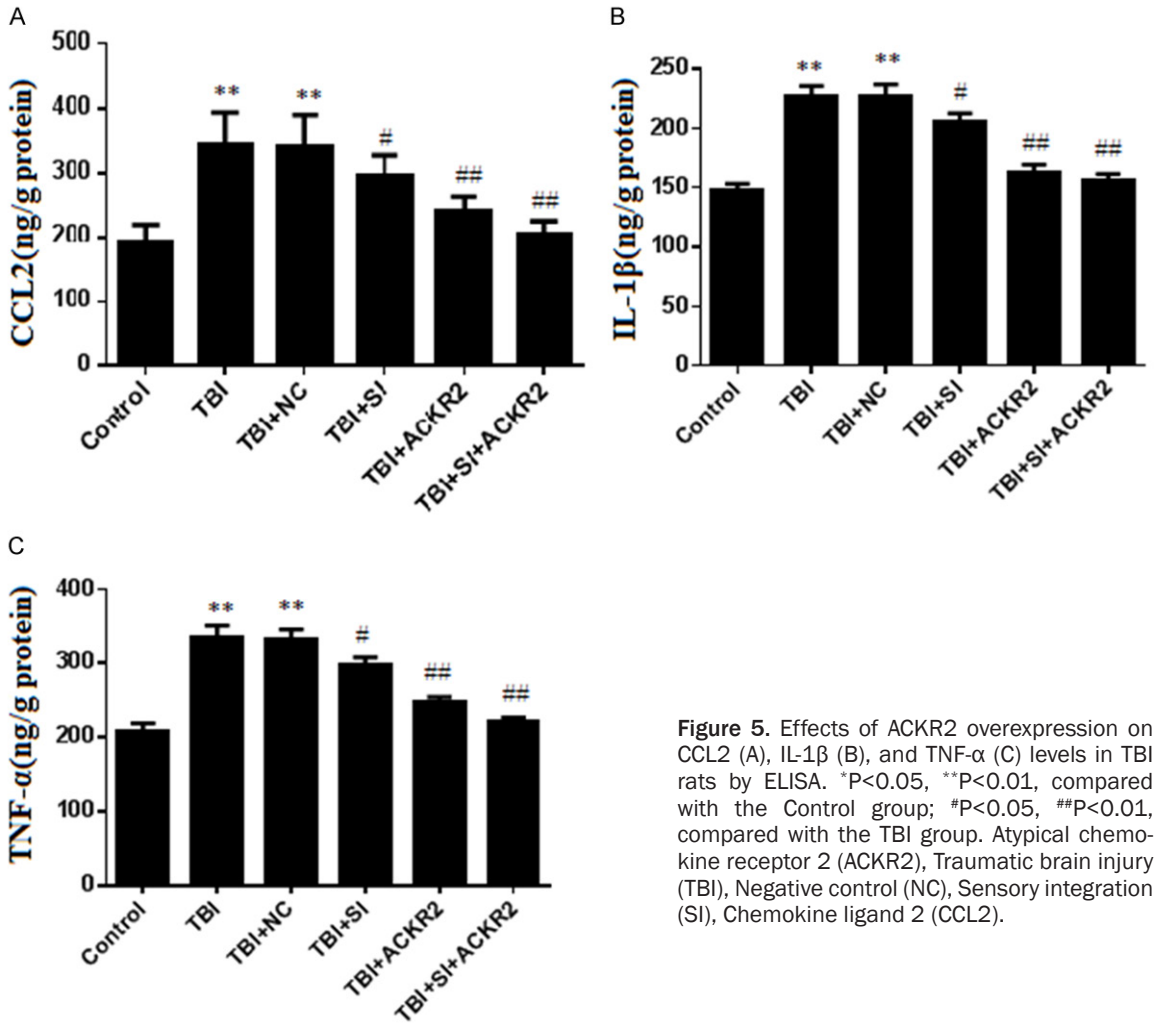

Figure 5. Effects of ACKR2 overexpression on CCL2 (A), IL-1β (B), and TNF-α (C) levels in TBI rats by ELISA. *P<0.05, **P<0.01, compared with the Control group; #P<0.05, ##P<0.01, compared with the TBI group. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI), Chemokine ligand 2 (CCL2).

Numerous peripheral inflammation models have demonstrated the ability of atypical chemokine receptor 2 (ACKR2) to resolve inflammation. ACKR2 may also play a different role in different organs, though the evidence is sparse and ambiguous at present [12, 19-22]. It is particularly unclear how ACKR2 functions in inflammatory conditions of the nervous system. In the brain, the data regarding the expression and function of ACKR2 is conflicting. In 1997, ACKR2 was first described in the mouse brain [23]. In two subsequent studies, ACKR2 immunoreactivity was demonstrated in primary human astrocytes [24, 25], whereas the other failed to confirm the presence of ACKR2 mRNA in cultured mouse astrocytes or detect fluorescently labeled chemokine ligand 2 uptake by these cells [26]. It has also been shown that chemokine networks in rodents and human victims with severe TBI are associated with increased brain pathology, inflammatory cell accumulation, neuronal death and sensorimotor deficits [27]. Accordingly, we hypothesized that ACKR2 expression would be down-regulated in rats with brain injury. In this study, we sought to define the potential role of the chemokine receptor ACKR2 and how TBI may influence its expression. In the TBI rat brain, we demonstrated for the first time a constitutive, low expression of ACKR2.

Chemokine ligand 2 (CCL2) plays a role in a variety of neurological diseases. Studies have shown that CCL2 deficiency leads to a mass accumulation of damaged microglia, and accelerates the progression of Alzheimer's disease (AD). There was a significant negative correlation between cerebrospinal fluid (CSF) and serum CCL2 levels and neurodegeneration in patients with mild cognitive impairment AD.

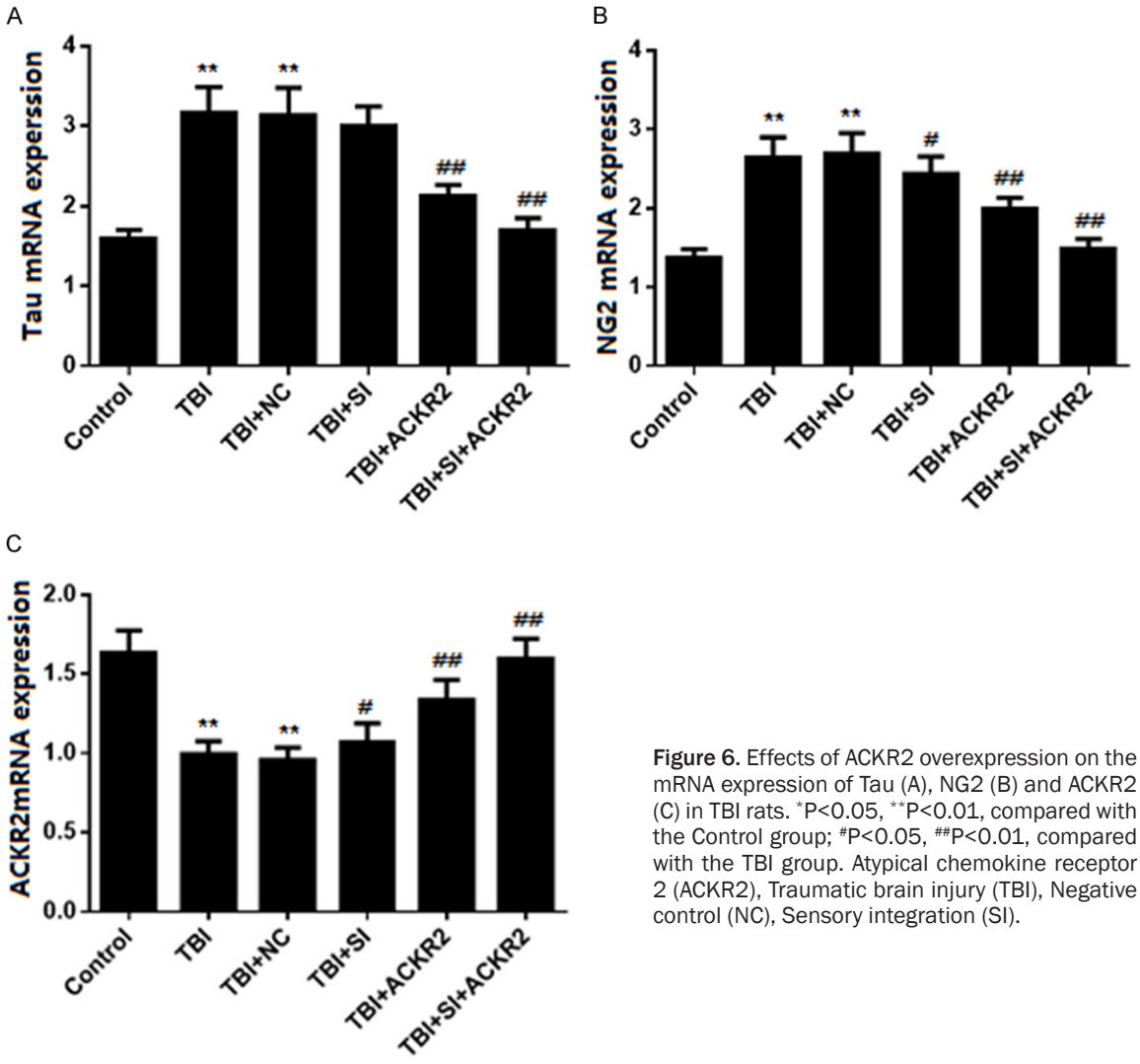

Figure 6. Effects of ACKR2 overexpression on the mRNA expression of Tau (A), NG2 (B) and ACKR2 (C) in TBI rats. $^{*}P<0.05$, $^{**}P<0.01$, compared with the Control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the TBI group. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI).

CCL2 levels are significantly elevated in the cerebrospinal fluid of Parkinson disease (PD) patients, which is positively correlated with the degree of depression in PD patients. Another study has shown that CCL2 can also increase the excitability of dopaminergic neurons in mice, increasing the level of dopamine and spontaneous activity in mice [28]. The above evidence suggests that CCL2 may be associated with cognitive impairment in neurological diseases. In addition, CCL2 can also induce microglia to produce TNF-α, and TNF-α can promote T lymphocytes to produce inflammatory cytokines, thus promoting the pathogenesis of inflammation and aggravating craniocerebral injury [29]. To further explore the relationship between ACKR2 and CCL2 in the pathogenesis of brain injury, we injected ACKR2 adenovirus overexpression vector through lateral ventricle localization of TBI rats, and observed a high expression of ACKR2 in TBI rats. Then the learning and memory abilities of TBI rats were observed by water maze. The result showed that ACKR2 overexpression combined with sensory integration training obviously promoted the learning and memory ability in TBI rats and improved the cognitive function of TBI rats. Transmission electron microscope showed that ACKR2 overexpression combined with sensory integration training recovered the morphology of the neurons. HE staining showed that ACKR2 overexpression combined with sensory integration training alleviated edematous and recovered the number and structure of neurons in the brain tissues. The protein expression of CCl2, TNF-α and IL-1β in brain tissue of TBI rats

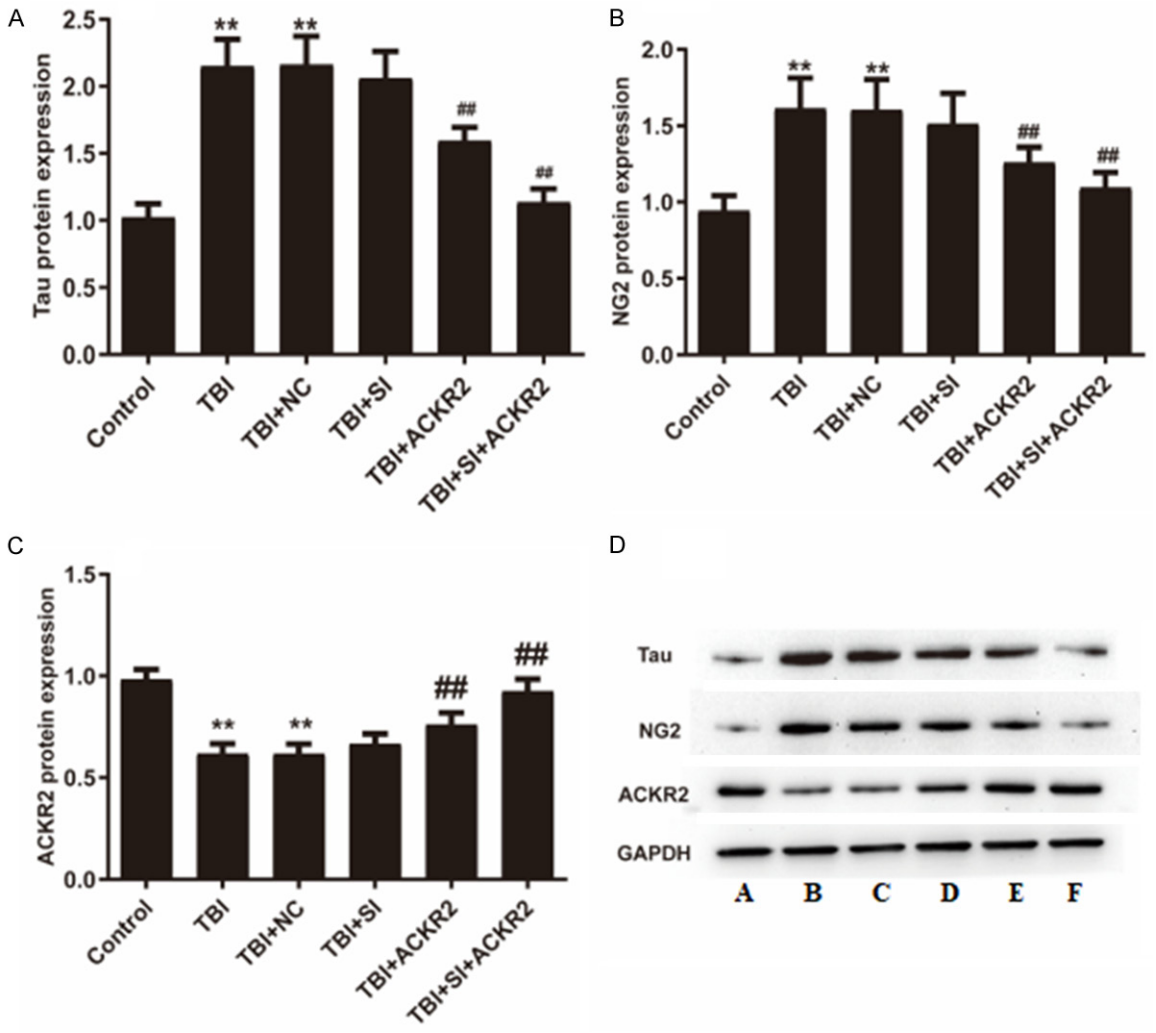

Figure 7. Effects of ACKR2 overexpression on the protein expression of Tau, NG2 and ACKR2 in TBI rats. A. Control group. B. TBI group. C. TBI+NC group. D. TBI+SI group. E. TBI+ACKR2 group. F. TBI+SI+ACKR2 group. $^{*}P<0.05$, $^{**}P<0.01$, compared with the Control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the TBI group. Atypical chemokine receptor 2 (ACKR2), Traumatic brain injury (TBI), Negative control (NC), Sensory integration (SI).

was significantly decreased, suggesting that ACKR2 may inhibit the release of inflammatory factors in brain tissue by regulating the expression of CCl2 and alleviate the inflammatory response caused by brain injury.

Microtubule-associated protein tau (Tau) protein is a kind of microtubule-related protein as the skeleton protein of neuronal cells, which maintains the stability of axon microtubules. Both primary and secondary brain injury can lead to massive nerve cell death, and a large quantity of Tau protein was released into the cerebrospinal fluid (CSF), resulting in a significant increase in the level of Tau protein in CSF. Thus, the total Tau concentration in the CSF is much higher than the baseline value after severe TBI. Studies have shown that the concentration of Tau protein in the CSF of patients with craniocerebral injury is 4000 times higher than that of patients with neurological diseases without traumatic brain injury and patients without neurological disorders [30]. In addition, other studies have shown that when the serum Tau protein concentration increased significantly at 6 months after severe TBI, the patients had a poor prognosis [31]. The results of this study showed that Tau protein expression was significantly increased in TBI rats. ACKR2 overexpression combined with sensory integration training could reduce the Tau protein expression.

NG2 is an important chondroitin sulfate proteoglycan in the central nervous system [32]. It has been found in studies of spinal cord trauma and cerebral ischemia that under physiological conditions, it is mainly expressed in the cerebral cortex, hippocampus, cerebellum, olfactory system and retina. Although their content in the brain is small, they play an important role in the development and maturation of nerve cells by regulating axon growth [33]. In animal experiments, phosphoproteoglycans (PC) and NG2 proteoglycans may be two of the important factors inhibiting axonal regeneration after traumatic brain injury (TBI). The increased expression of NG2 and the number of ng2-positive cells in neural tissues and cerebral ischemic penumbra after trauma indicates that oligodendrocyte progenitors begin to proliferate in neural tissues after injury, which may replenish the dead oligodendrocytes and prepare for the formation of myelination in neuronal axons [34, 35]. In the adult brain, NG2-glia represents a cell population that responds to injury [36]. Our results showed that NG2 protein expression was significantly increased in TBI rats. ACKR2 overexpression combined with sensory integration training could reduce the NG2 protein expression.

In conclusion, ACKR2 is associated with cognitive impairment in traumatic brain injury in young rats, and ACKR2 can clear inflammatory CC-chemokines, release interleukin-1β and tumor necrosis factor-α and regulate the expression of related proteins such as Tau and NG2.

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Disclosure of conflict of interest

None.

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