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Original Article

Elevated hippocampal CD24 in astrocytes participates in neural regeneration possibly via activating SHP2/ERK pathway after experimental traumatic brain injury in mice

Xuan Gao[1*], Han Wang[1,2*], Yong-Yue Gao[1], Xiao-Ming Zhou[3], Tao Tao[4], Guang-Jie Liu[1], Yan Zhou[1], Wei Li[1], Chun-Hua Hang[1]

[1]Department of Neurosurgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China; [2]Department of Neurosurgery, Jinling Hospital, The First School of Medicine, Southern Medical University (Guangzhou), Nanjing, China; [3]Department of Neurosurgery, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China; [4]Department of Neurosurgery, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, China. [*]Equal contributors.

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Abstract: Massive neuron loss is the key reason for poor prognoses in patients with traumatic brain injury (TBI), and astrocytes function as nutrition-providing neurons. Therefore, researchers must determine the potential role of astrocytes in neural regeneration after TBI. Our previous studies established that upregulating CD24 in the hippocampus might improve cognitive functions after TBI. However, whether CD24 in hippocampal astrocytes is involved in neural regeneration after TBI remains unknown. Therefore, we detected the CD24 expression in the ipsilateral hippocampus via western blot and quantitative real-time PCR. We further investigated the CD24 expression patterns in hippocampal astrocytes via immunofluorescence staining. We then injected adeno-associated virus-Gfa2-siRNA-CD24 (AAV-CD24) into the astrocytes to downregulate CD24 and analyzed the related cellular signals. Golgi-Cox staining and the growth associated protein-43 (GAP43) level were used to observe neuronal morphology and neural regeneration around the astrocytes in the ipsilateral hippocampus, and the Morris water maze test was used to assess neural functional recovery. The CD24 protein and mRNA levels in the cornu ammonis and dentate gyrus regions of the ipsilateral hippocampus were elevated after TBI, and high CD24 expression was widespread in the hippocampal astrocytes after TBI. Specific inhibition of CD24 in the hippocampal astrocytes interfered with the activation of Src homology region 2 containing protein tyrosine phosphatase 2 (SHP2) and extracellular signal regulated kinase (ERK), shortened the neuronal dendritic spines, decreased the GAP43 level and impaired the cognitive functions of the TBI-model mice. These results revealed that elevated hippocampal CD24 in astrocytes participated in neural regeneration in mice after TBI, possibly by activating the SHP2/ERK pathway.

Keywords: CD24, astrocytes, traumatic brain injury, SHP2/ERK, neural regeneration

Introduction

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, seriously threatening people's lives and affecting their quality of life [1, 2]. TBI causes neurophysiological damage and impairs neurological function after the head is impacted by mechanical forces. Because TBI is catastrophic, many clinicians and medical researchers are investigating it [3, 4]. Massive neuronal losses can lead to a poor prognosis for patients with TBI, and the pathogenesis involves a complex network of neural damage, repair and regeneration [5-7]. Therefore, exploring the TBI process is of vital importance to find optimal therapies and improve patients' long-term neurological functioning after TBI.

Springer first identified the cluster of differentiation 24 (CD24) in 1978 after producing xenogenic rat anti-mouse antibodies. Because CD24 is heat resistant, it was initially referred to as a heat-stable antigen [8]. CD24 plays an impor-

tant role in cellular communication in the central nervous system and functions as a neural cell surface antigen [9-11]. Our previous studies showed that elevated hippocampal CD24 expression was associated with neural regeneration after TBI [12, 13]. Furthermore, astrocytes influence their extracellular environment, modulate neighboring cells, and provide nutrition for neurons [14]. Therefore, we hypothesized that CD24 in hippocampal astrocytes may play a role in neural regeneration after TBI. Hence, in the current study, we analyzed the expression patterns of hippocampal CD24 in astrocytes and evaluated the potential role of CD24 in neural regeneration after TBI.

Materials and methods

Animals

All male adult C57BL/6 mice (25 g-30 g) purchased from Experimental Animal Center of Drum Tower Hospital, Nanjing, China. Ample food and water were provided and rat was kept in specific pathogen-free (SPF) and comfortable conditions (12-h light/dark cycle, temperature at 25°C, and humidity at 65%) throughout the experiment. The experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee at Drum Tower Hospital and conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

TBI model

Experimental TBI model used in this study was induced by a modified instrument as described in previous experiments [12, 13]. Briefly, 1.5% isoflurane inhalation (RWD Life Science, Shenzhen) was used to anesthetize mice for induction. After disinfection, a 2.0 cm midline scalp incisions was made to expose the skull. Afterwards, the weight-drop device with a 333 g of weight comprising a metal rod 3 mm in diameter and 5 mm in length dropped from a height of 2.5 cm directly onto the exposed skull, causing a focal trauma on the left hemisphere 2 mm lateral to the midline in the midcoronal plane. Finally, the scalp wound was thoroughly disinfected and closed. Sham groups were gone through the same process without injury. All mice were returned to the cages and keep a comfortable environment.

Experimental design

Experimental design 1: To examine the CD24 expression and distribution in the hippocampus of mice after TBI. All 36 mice were randomly assigned to six groups: sham group and TBI groups (3, 7, 10, 14, 28 d) (n = 6 each). Six mice in each group were used for western blotting and qPCR.

Experimental design 2: To explore the CD24 function and potential mechanism in the hippocampus of mice after TBI. All 48 mice were randomly assigned to four groups: sham group, TBI group, TBI + vehicle group and TBI + AAV group (n = 12 each). The experimental TBI was established at 3 d after AAV injection. All mice were sacrificed on day 7 after TBI. Six mice in each group were randomly selected for western blotting and qPCR, and the rest of mice were used for immunofluorescence staining.

Experimental design 3: To explore the influences of CD24 in hippocampal astrocytes for neurons and cognitive functions of mice after TBI. All 24 mice were randomly assigned to four groups: sham group, TBI group, TBI + vehicle group and TBI + AAV group (n = 6 each). The experimental TBI was established at 3 d after AAV injection. All mice would receive 7 days of consecutive training and 1 day of probe trails at 3 d after TBI. Then all mice would be sacrificed for Golgi-Cox staining after probe trails.

Adeno-associated virus construction and injection

Downregulation of CD24 was achieved by transfection of adeno-associated virus (AAV). To establish and maintain the specific downregulation of CD24, the AAV-Gfa2-siRNA-CD24 (AAV-CD24) was designed by HANBIO (Shang-Hai, China). Meanwhile, the AAV-Gfa2-NC (AAV-NC) was used as negative control. The CD24 siRNA sequence is 5'-AAATATTCTGGTTACCG-GGAACGG-3' and NC sequence is 5'-TTCTCC-GAACGTGTCACGT-3'. The experimental TBI was established at 3 d after AAV injection.

Western blot analysis

The ipsilateral hippocampus was obtained after intracardiac perfusion with 0.9% refrigerated saline. Then, these tissues were lysed with RIPA (Thermo Scientific, USA) with prote-

ase inhibitor (Roche, Switzerland) and 1% phosphatase inhibitor (Sigma, USA). After the same mass of protein was quantified, it was loaded onto SDS-PAGE gels for separation and then transferred to a PVDF membrane. The membrane was blocked in 5% skimmed milk for 1 h at room temperature and incubated with primary antibody against CD24 (ab640-64), Src homology region 2 containing protein tyrosine phosphatase 2 (SHP2) (#3397), p-SHP2 (Tyr542) (#3751), extracellular signal Regulated Kinase (ERK) (#4695), p-ERK (Thr202/Tyr204) (#4370), GAPDH (#5174), GAP43 (1:1000; ab16053, Abcam, Cambridge, MA, USA) and β-actin (#3700) (1:1000; Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. After washed 3 times for 10 minutes with 1 × trisbuffered saline and Tween 20, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (ab7097 anti-rat, #7074 anti-rabbit, #7076 anti-mouse) for 1 h at room temperature. Finally, following 5 × 5-min washing with PBST, detection was performed by Immobilon Western Chemiluminescent HRP substrate (Millipore Sigma, Burlington, MA, USA) according to the manufacturer's instruction. Pictures were analysed with Image J.

Quantitative real-time PCR

Total RNA was extracted with the Trizol (Invitrogen, USA) from the ipsilateral hippocampus. cDNA was reverse transcribed from mRNA with reverse transcription mix (R302-01, Vazyme, Nanjing) after removal of genomic DNA. qPCR was performed with SYBER Green mix (Roche, Switzerland) with the assistance of the PCR system (Applied Biosystems, USA). The results were analysed by the 2-ΔΔCt method, while GAPDH RNA was used for normalization. The CD24 forward and reverse primers were 5'-GTTGCTGCTTCTGGCACTG-3', 5'-GGTAGCGTTACTTGGATTTGG-3', GAP43 forward and reverse primers were 5'-GGCCGCAACCAAAATTCAGG-3', 5'-CGGCAGTAGTGGTGCCTTC-3' and GAPDH forward and reverse primers were 5'-AATGGATTTGGACGCATTGGT-3', 5'-TTTGCACTGGTACGTGTTGAT-3.

Immunofluorescence staining

Firstly, the brain frozen sections were fixed in 4% paraformaldehyde, permeability with 0.3% Triton X-100, and blocked with Immunostaining blocking fluid (Epizyme, Shanghai). Next, these sections were incubated with primary antibody against CD24 (1:200, ab64064, Abcam), p-SHP2 (Tyr542) (#3751) and p-ERK (Thr202/Tyr204) (#4370) (1:100; Cell Signaling Technology) overnight at 4. After 3 × 5-min washing with PBS, the sections were incubated with corresponding secondary antibodies for an hour at room temperature. Finally, the pictures were acquired by a confocal laser scanning microscope (FluoView FV10i, Olympus, Japan). Immunofluorescence cell count and intensity were analysed by Image J.

Golgi-Cox staining

After effective anesthesia, 20 ml PBS was used to perfuse by transcending perfusion. According to the manufacturer's instructions, FD Rapid GolgiStain™ Kit (FD NeuroTechnologies, Columbia MD) was used to remove the brain. The coronal sections were cutting using a Leica VT1000 S Vibrating-blade vibration. After proper section in artificial cerebrospinal fluid (240 mM sucrose, 3.3 mM KCl, 26 mM $NaHCO_3$, 1.3 mM $MgSO_4 \cdot 7H_2O$, 1.23 mM NaH_2PO_4, 11 mM D-glucose, and 1.8 mM $CaCl_2$), these sections were mounted on gelatin-coated slides with a drop of 'C' solution from the FD Rapid GolgiStain™ Kit. After mounting sections on the slide, the remaining 'C' solution was wiped away with a strip of filter paper to ensure that the slides totally dried and that the sections remained attached during the staining process. The slides were dried in the dark in a fume hood for at least 24 h prior to staining.

Morris water maze test

The MWM test was used to evaluate cognitive functions. Briefly, MWM apparatus consists of a custom-built circular pool (1.2 m diameters and 30 cm height) with white non-reflective inner surface. The pool was filled with water (25-27°C) up to the level of 24 cm. In addition to a platform with a diameter of 12 cm was located in a random quadrant of the maze. Visual cues were directly placed on the inside of the pool wall near the platform. Each animal was trained for 7 days, with 3 trials per day consecutively. Whole tests were under monitored with the video tracking system attached to the computer with WM software (NI-IMAQ,

Coulbourn Instruments. USA) for recording various parameters.

Statistical analysis

All data were expressed as the means ± SD. Statistical comparisons were performed with SPSS. The latency and swimming speed in the acquisition phase was analysed by two-way repeated analysis of variance (ANOVA). The rest datas were analysed by one-way ANOVA. Values of $P < 0.05$ were considered statistically significant.

Results

CD24 in hippocampal astrocytes was upregulated in experimental TBI-model mice

CD24 protein and mRNA expressions were analyzed via western blot and qPCR (**Figure 1A**). The CD24 protein level was significantly elevated at 3 d ($P < 0.05$) and peaked around 7 d after TBI ($P < 0.05$). After day 10, the CD24 expression returned to near baseline. Similar to the changes in protein levels, the CD24 mRNA levels were also elevated at 3 d ($P < 0.05$) and peaked at 7 d ($P < 0.05$). Although the CD24 mRNA levels remained higher after 10 d than did those in the sham group, they did not statistically differ ($P > 0.05$). To explore the CD24 expression patterns, the cornu ammonis (C1) and dentate gyrus (DG) regions were stained with immunofluorescence markers. CD24 was widely expressed in astrocytes in both the C1 and DG regions (**Figure 1B**). The levels of hippocampal CD24 and the astrocyte activation-specific marker, GFAP, were both elevated after TBI ($P < 0.05$), and the CD24/GFAP ratio was much higher after TBI ($P < 0.05$; **Figure 1B**).

Adeno-associated virus (AAV) downregulated CD24 expression in hippocampal astrocytes

To investigate the effects of hippocampal CD24 in astrocytes after TBI, we suppressed hippocampal CD24 in astrocytes via AAV injection in TBI-model mice. As expected, AAV markedly downregulated CD24 protein and mRNA expressions in the C1 and DG regions ($P < 0.05$; **Figure 2A**, **2B**). No significant difference was found between the TBI and TBI + vehicle groups ($P > 0.05$; **Figure 2B**). Immunofluorescence staining revealed that CD24 was markedly downregulated in the astrocytes in both the C1 and DG regions after AAV injection compared with those in the TBI + vehicle group ($P < 0.05$). Although some hippocampal astrocytes were killed in the TBI + AAV group ($P > 0.05$), CD24 expression was almost completely suppressed in the live hippocampal astrocytes ($P < 0.05$; **Figure 3**).

Inhibition of hippocampal CD24 in astrocytes suppressed SHP2/ERK pathway activation

SHP2, p-SHP2, ERK, and p-ERK protein expressions were assessed via western blotting. The SHP2/ERK pathway was activated after TBI ($P < 0.05$), and the SHP2 and ERK phosphorylation levels decreased after inhibiting hippocampal CD24 in astrocytes post-TBI ($P < 0.05$; **Figures 2C**, **4**). Thus, inhibition of CD24 remitted the SHP2/ERK pathway activation.

Downregulation of hippocampal CD24 in astrocytes decreased neural regenerationin TBI-model mice

Golgi-Cox staining showed the neuronal morphology in the sham, TBI, TBI + vehicle and TBI + AAV groups (**Figure 5A**). The dendritic spines of the neurons were shortened after TBI ($P < 0.05$), and the dendritic spines in the TBI + AAV group were shorter than those in the TBI + vehicle group ($P < 0.05$). GAP43 protein and mRNA expressions were analyzed via western blot and qPCR (**Figure 5B**). The GAP43 protein and mRNA levels were significantly decreased after TBI ($P < 0.05$), and the GAP43 protein and mRNA levels in the TBI + AAV group were less than those in the TBI + vehicle group ($P < 0.05$).

Downregulation of hippocampal CD24 in astrocytes harmed spatial learning and memory behavior in TBI-model mice

Morris water maze tests showed that the average escape latencies in all four groups were shortened with training, and a significant advantage was noted on the training day in the sham group compared with that of the TBI, TBI + vehicle and TBI + AAV groups ($P < 0.05$; **Figure 6A**). During the training period, swimming speeds among the four groups did not significantly differ ($P > 0.05$; **Figure 6B**), nor did they differ among the four groups in the probe trials ($P > 0.05$; **Figure 6C**). However, the percentage

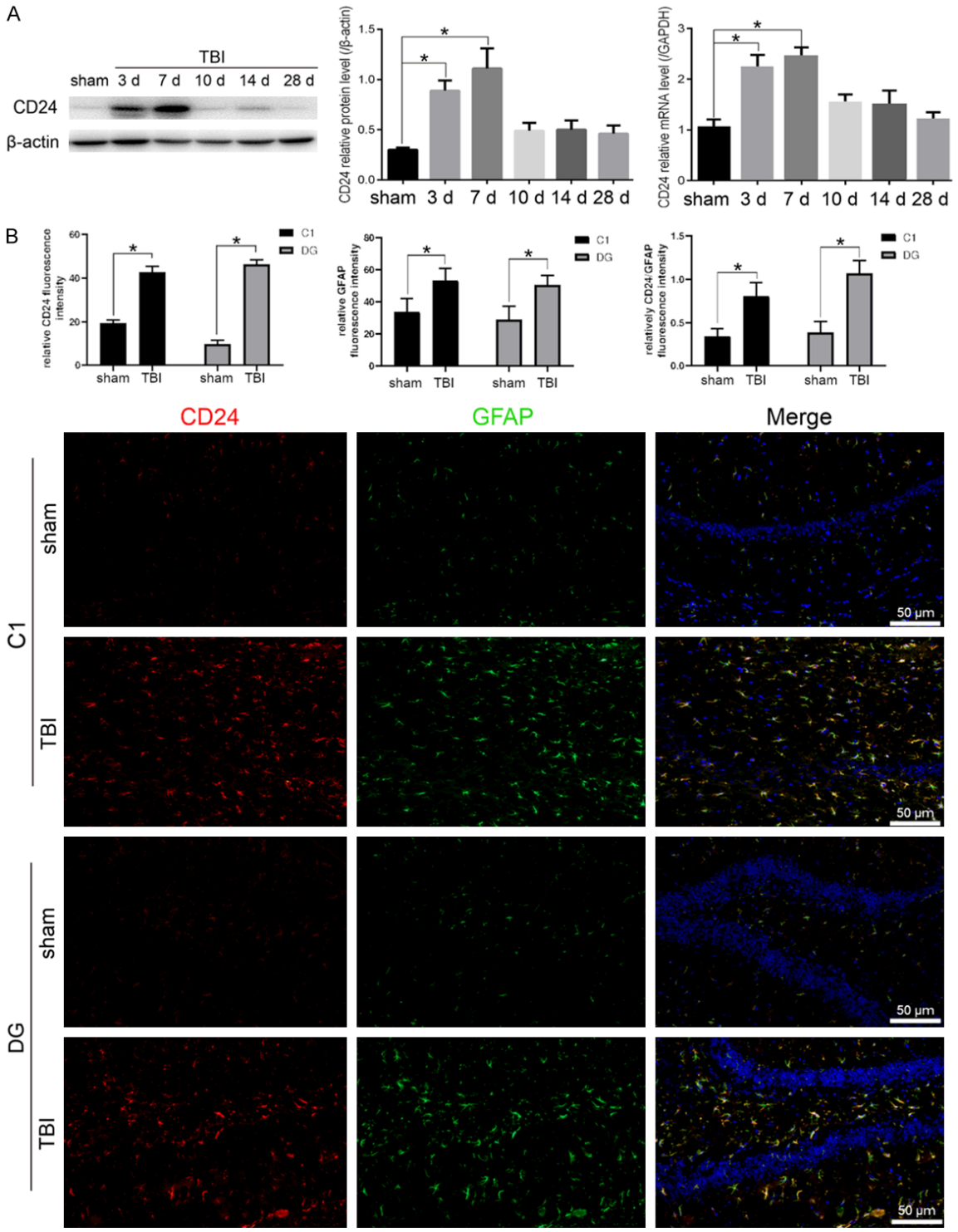

Figure 1. CD24 expression in hippocampal after experimental TBI in vivo. A. Western blot assay of CD24 expression at each time point (3, 7, 10, 14, 28 d) after TBI. Quantitative analysis of western blot and quantitative real-time PCR results showed that CD24 protein and mRNA both peaked at 7 d after TBI. B. Double immunofluorescence staining showed that CD24 reflected in astrocytes of the ipsilateral C1 and DG (CD24 = red, GFAP = green, DAPI = blue). Semiquantitative analysis showed that the number of CD24-positive cells, GFAP-positive cells and the CD24/GFAP ratio were all increased after TBI. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. sham or indicated groups (n = 6 in each group).

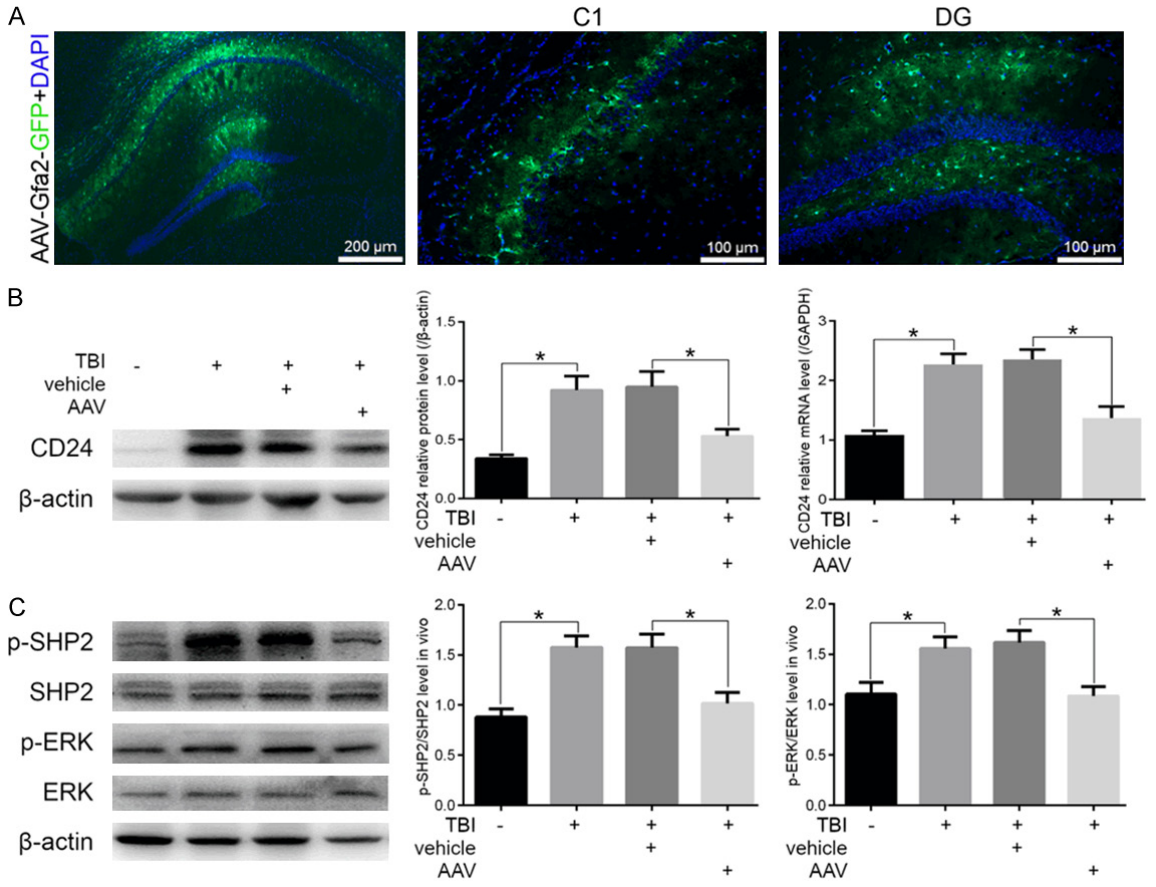

Figure 2. Adeno-associated virus (AAV) downregulated CD24 expression in hippocampal astrocytes. A. Double immunofluorescence staining showed that AAV was injected in C1 and DG. B. Western blot assay of CD24 expression in sham, TBI, TBI + vehicle and TBI + AAV groups. Quantitative analysis of western blot and quantitative real-time PCR results showed that AAV markedly downregulated CD24 protein and mRNA expressions. C. Western blot assay of SHP2, p-SHP2, ERK, p-ERK and β-actin, the results showed that the ratio of p-SHP2/SHP2 and p-ERK/ERK were both elevated after TBI and decreased after AAV injection. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. sham or indicated groups (n = 6 in each group).

of time in the correct quadrant and the total number of platform crossings statistically differed among the four groups ($P < 0.05$; **Figure 6D**, **6E**). The percentage of time in the correct quadrant was significantly shorter in the TBI group than in the sham group ($P < 0.05$), and the performance of the TBI + AAV group was much worse than that of the TBI + vehicle group ($P < 0.05$). Results for the total number of platform crossings were consistent with those for the percentage of time in the correct quadrant ($P < 0.05$).

Discussion

The central nervous system is fragile and complex. Being stricken by violent forces can have detrimental consequences such as amnesia, a persistent vegetative state and dementia. The hippocampus is receiving attention in TBI research because it is essential for memory, learning and cognition [15]. Studies have shown that the hippocampus is a C-shaped structure composed of a head, body and tail, two rolled-up laminae, the cornu ammonis (CA1-CA4) and the dentate gyrus [16]. The hippocampus is related to cognitive function after TBI [13]; therefore, determining the damage to the hippocampus after TBI is crucial for therapy and recovery. Thus, we analyzed experimental TBI models of the ipsilateral hippocampus for further scientific research.

In our previous studies, we found that hippocampal CD24 might be closely related to cognitive functions after TBI [13]. Better understand-

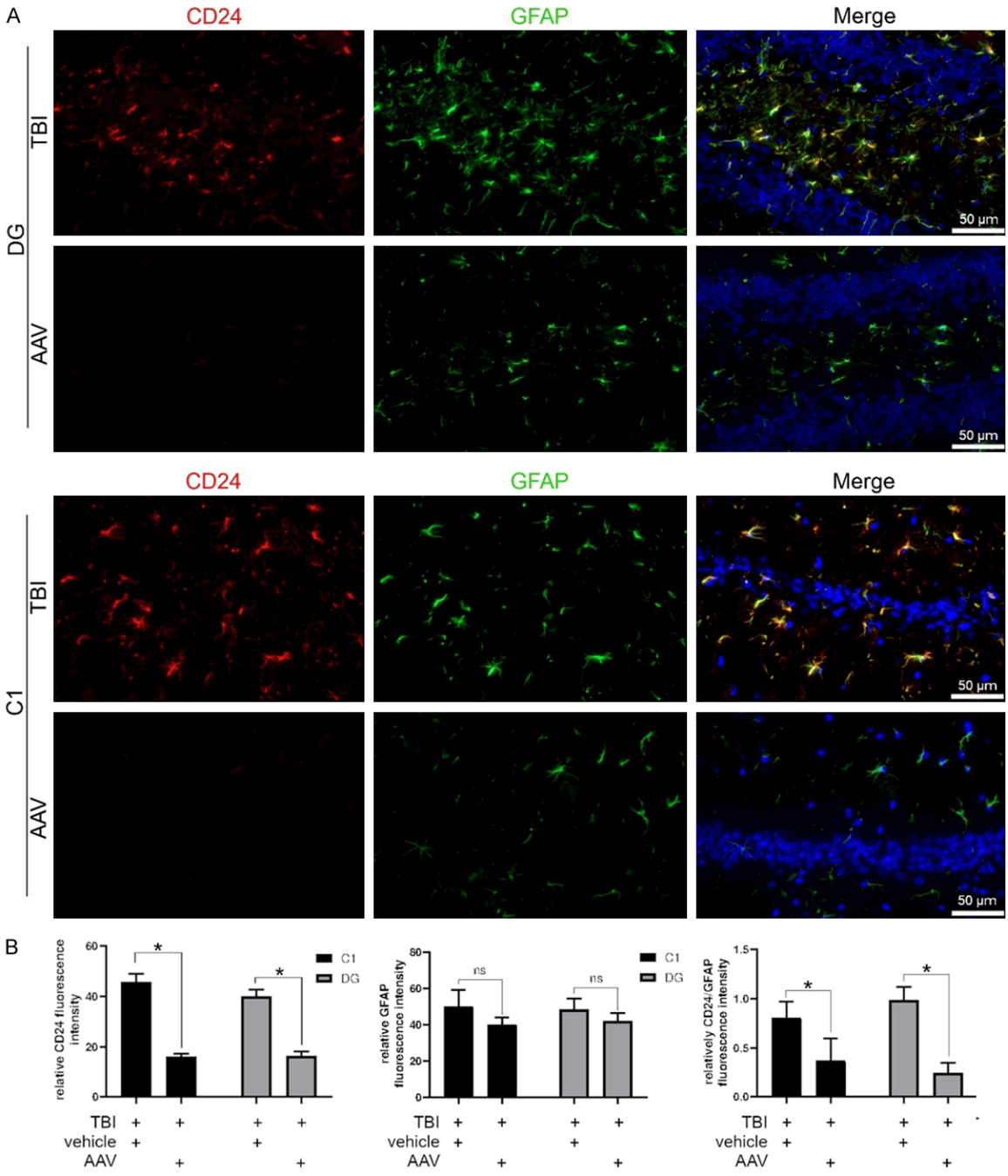

Figure 3. CD24 expression in hippocampus after AAV injection. A. Representative immunofluorescence staining for CD24 and GFAP in the ipsilateral C1 and DG after AAV injection (CD24 = red, GFAP = green, DAPI = blue). B. Semiquantitative analysis showed that the number of CD24-positive cells and the CD24/GFAP ratio were decreased while the number of GFAP-positive cells almost unchanged after AAV injection. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. indicated group (n = 6 in each group).

ing the CD24 molecular structure contributes to deeper investigations of the diverse functions and signaling mechanisms of CD24. CD24 is conserved across many mammalian species and most organs for adaptive immune responses [17-19]. In 1990, the mouse CD24 gene was found to encode a small protein consisting of only 27 amino acids [20]. The mouse CD24 gene is located on chromosome 10, and two intronless CD24 retroposons are located

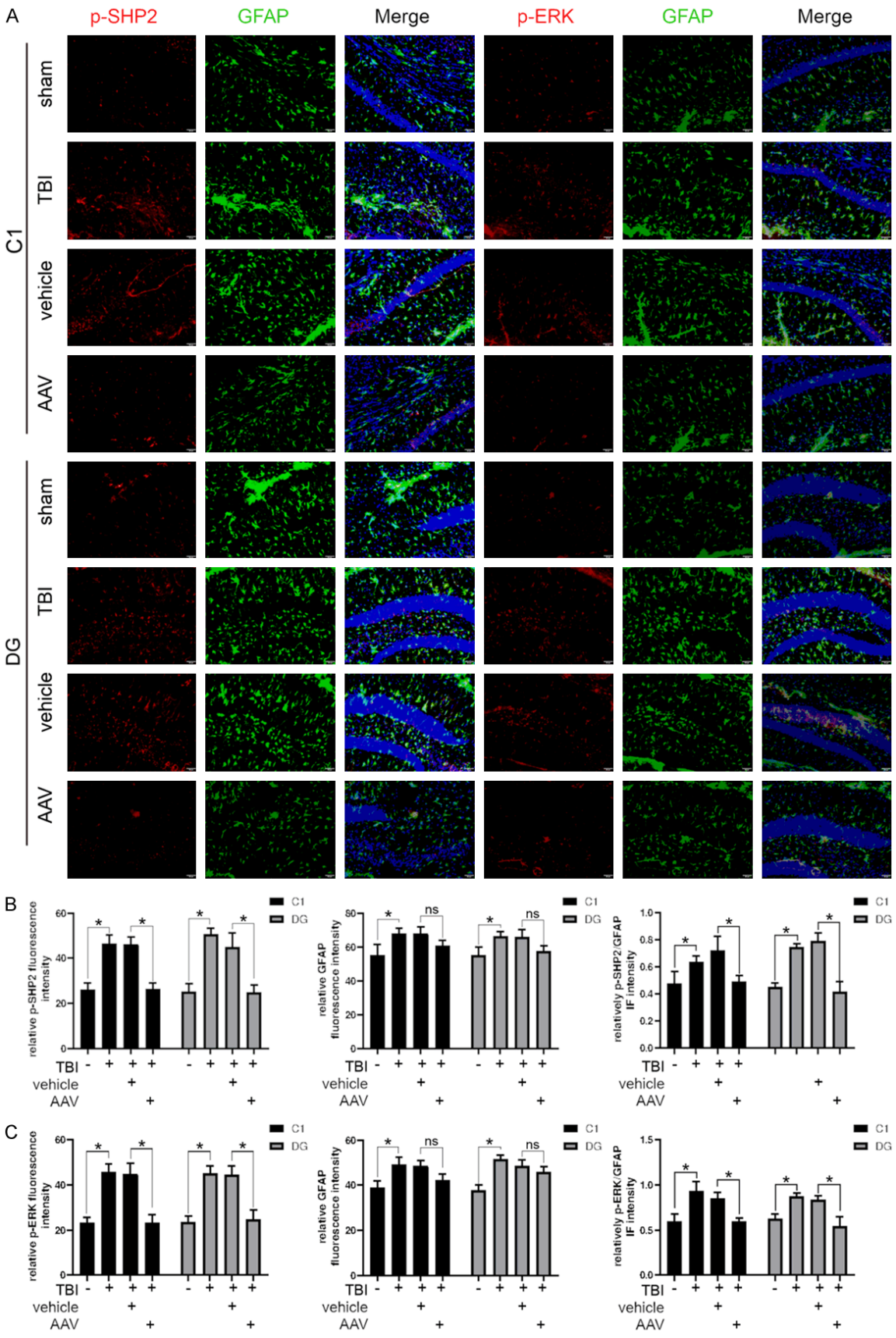
A
p-SHP2
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Figure 4. The SHP2 and ERK phosphorylation levels in hippocampus after experimental TBI and AAV injection. A. Representative immunofluorescence staining for p-SHP2 and p-ERK in the ipsilateral C1 and DG (p-SHP2/p-ERK = red, GFAP = green, DAPI = blue). B. Semiquantitative analysis of the number of p-SHP2-positive cells, the number of GFAP-positive cells and the p-SHP2/GFAP ratio in the sham, TBI, TBI + vehicle and TBI + AAV groups. C. Semiquantitative analysis of the number of p-ERK-positive cells, the number of GFAP-positive cells and the p-ERK/GFAP ratio in the sham, TBI, TBI + vehicle and TBI + AAV groups. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. indicated group (n = 6 in each group) Bar = 50 um.

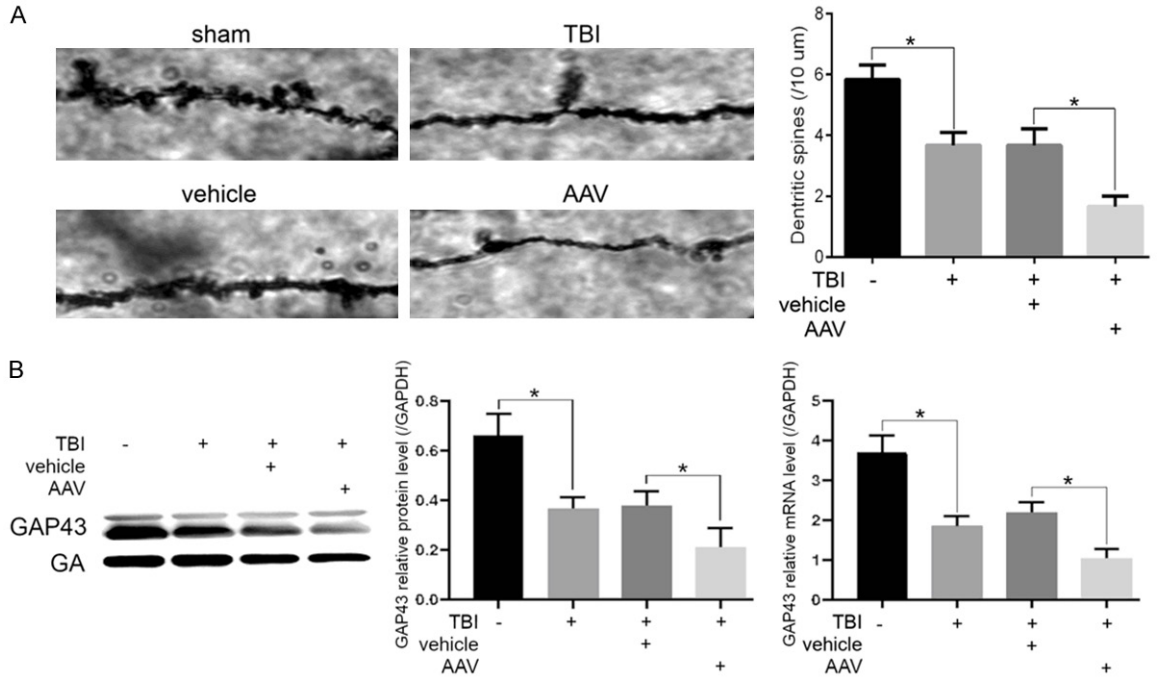

Figure 5. Downregulation of CD24 in the hippocampal astrocytes decreased neural regeneration. A. Examples of Golgi-Cox stained neurons showed that neurons morphology in sham, TBI, TBI + vehicle and TBI + AAV groups. Semiquantitative analysis showed the spines were shortened after TBI and the spines in TBI + AVV group were shorter. B. Western blot assay of GAP43 expression in sham, TBI, TBI + vehicle and TBI + AAV groups. Quantitative analysis of western blot and quantitative real-time PCR results showed that AAV markedly downregulated GAP43 protein and mRNA expressions. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. sham group or indicated group (n = 6 in each group).

on chromosomes 8 and 14 [21]. Later, the human CD24 gene was found on chromosomes 1, 6, 15, 20 and Y [22]. Except for mRNA, the human CD24 gene encodes a transcript for an open reading frame (0.24 kb) and a long 3'-untranslated region (1.8 kb) to regulate mRNA stability [23, 24]. After translation, the signal peptide is removed, a glycosyl phosphatidylinositol anchor is attached, and specific glycan moieties are incorporated. These modifications remove two-thirds of the original amino acids, resulting in a mature CD24 peptide of 32 residues in humans and 27 residues in the mouse ortholog [25]. CD24 from different tissues and cells is also changeable. Ranging from 20-70 kDa, CD24 is highly variable and cell-type-dependent due to its glycosylation [25-28].

Much research has been conducted on CD24. CD24 plays a significant role in adaptive immune responses, inflammation, autoimmunity, cancer and nervous system diseases [9, 29]. Adaptive immune responses require CD24 for optimal homeostatic T-cell proliferation in hosts with lymphopenia, and CD24 deficiency can suppress both CD4 and CD8 T-cell responses [30, 31]. During inflammation, CD24 is reported to be associated with a variety of damage associated molecular patterns (DAMPs) and can selectively repress host responses to tissue injury [32, 33]. In autoimmunity, CD24 expressed in the microglia and astrocytes may promote pathogenic T-cell activation and proliferation [34]. CD24 polymorphisms are associated with progression of autoimmune diseases [35].

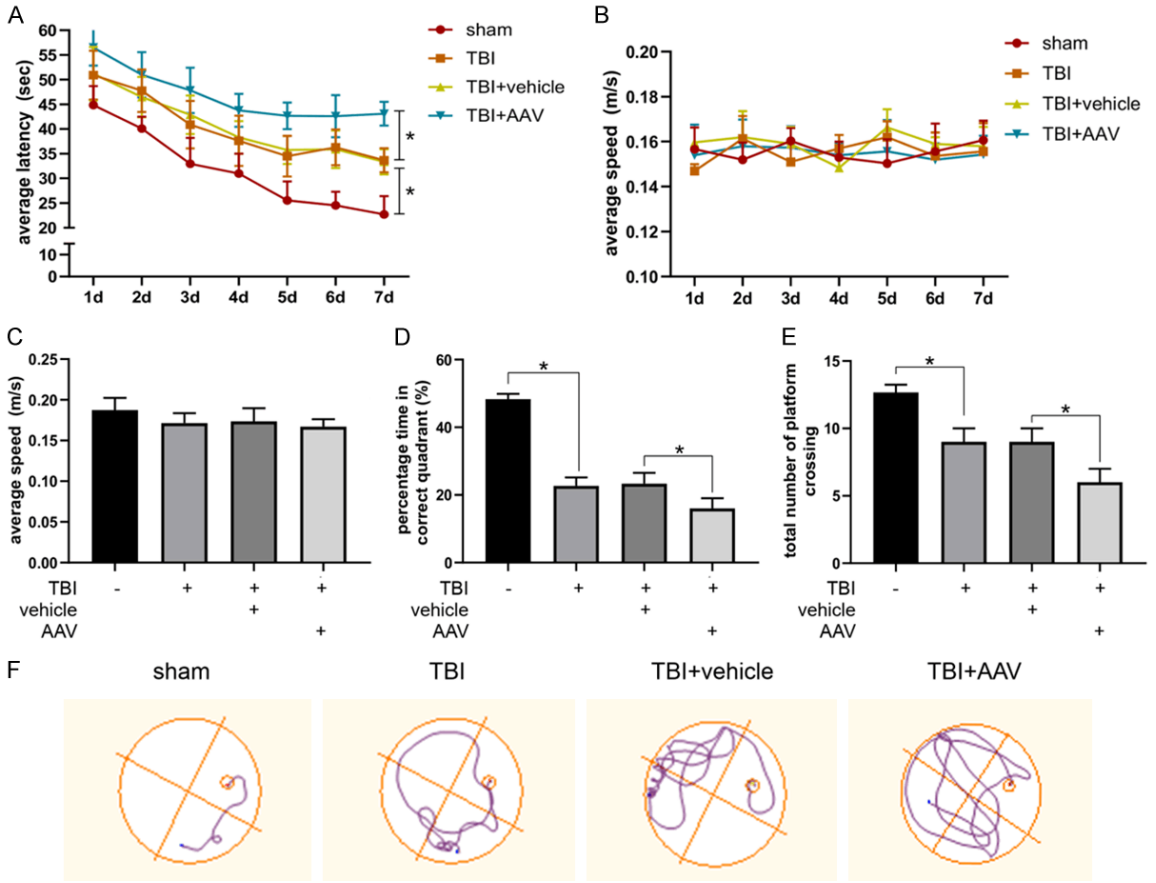

Figure 6. Downregulation of CD24 in the hippocampal astrocytes harmed the performance on the Morris water maze test. (A) Average escape latency was shortened with training. Swimming speed did not significantly differ among the four groups during (B) the acquisition trials and (C) the probe trials. (D) Percentage of time spent in the correct quadrant and (E) Total number of platform site crossings statistically differed among the four groups during the probe trials. (F) Representative swimming path of four groups in the probe trials. Bars represent the mean ± SD. $^{ns}P > 0.05$, $^{*}P < 0.05$. vs. sham group or indicated group (n = 6 in each group).

Many studies have been conducted on CD24 in cancer cells. Considering the role of CD24 in intercellular communications, numerous interactions occur between CD24 and various cellular signaling pathways in cancer cells [29]. In various cancer cells, CD24 expression is broadly elevated, and CD24 can either promote or inhibit cell signaling pathways. CD24 promotes MAPK [36-42], Src [43, 44], EGFR [45] and TGF-β [46] signaling activation and inhibits NF-κB [12, 47] and notch and hedgehog signaling activation [48]. In the central nervous system, CD24 can play an important role in cellular communication and function as a neural cell surface antigen [10, 11, 33].

CD24 in the hippocampus likely participates in pathological changes after TBI. Our previous studies showed that downregulation of CD24 in the hippocampus might harm cognitive functions after TBI [13]. However, the specific distribution of CD24 in the hippocampus after TBI remains unknown. Regarding brain injuries, neuronal damage is typically considered the most important, and astrocytes are often ignored. Neurons dominate the research among TBI studies. Studies have shown that astrocytes are activated and play significant roles in neurofunctional prognosis after TBI [49]. The current study showed that high CD24 expression is widespread in hippocampal astrocytes. Astrocytes are the most abundant cells in the central nervous system, and their functions and morphologies differ depending on their locations and stages [50]. Astrocytes were once regarded as useless and thought to be merely space-filling support cells in the central nervous system [14]. However, as research

progressed, astrocytes were gradually found to have ample receptors, ion channels, and second messenger systems that assist them in influencing their extracellular environment and effectively modulating neighboring cells. Astrocytes are now recognized to play important roles in blood brain barrier formation and maintenance, neurotransmission, glutamate synthesis, homeostasis, and synaptic development and plasticity [14, 51]. Therefore, we tested CD24 expression levels in hippocampal astrocytes. We found that CD24 existed throughout hippocampal astrocytes and was markedly elevated after TBI. Although astrocytes were also activated after TBI, the CD24/GFAP ratio remained much higher.

In our current experiment, AAV was injected into experimental TBI-model mouse brains to restrain CD24 expression in hippocampal astrocytes. AAV killed a few astrocytes owing to the side effects of the virus; however, CD24 expression was almost completely suppressed in the live hippocampal astrocytes. Searching data revealed SHP2/ERK signaling expressions, and we found that TBI promoted SHP2 and ERK phosphorylation, whereas AAV inhibited them. SHP2 contains a central phosphotyrosine phosphatase (PTP) domain, two N-terminally located Src homology 2 domains (N-SH2 and C-SH2) and a C-terminal tail with tyrosyl phosphorylation [52, 53]. A unique characteristic of SHP2 is that one of the rare PTPs promotes intracellular signaling pathway activation rather than downregulation [54]. To our knowledge, it can stimulate the Ras/ERK MAP kinase pathway in response to diverse agonists. The Ras/ERK MAP kinase pathway is a major signaling cascade that modulates many cell fates, including proliferation, differentiation and death mobilized by a broad range of membrane receptors [52, 55]. SHP2 is essential for fully activating the ERK MAP kinase pathway in most cases, and studies have shown that the SHP2/ERK pathway is evolutionarily conserved [52].

Considering the relationship between astrocytes and neurons, Golgi-Cox staining and detection of the GAP43 level were conducted to observe the neural regeneration after hippocampal CD24 suppression in astrocytes. A single astrocyte can contact on the order of 100,000 synapses, and astrocytes can sense synaptic activity and modify it [56, 57]. Finally, we applied behavioral methods to detect mouse learning and memory levels. From these results and our previous works, we concluded that CD24 elevation in hippocampal astrocytes likely modifies neurons and participates in neural regeneration after TBI by activating the SHP2/ERK pathway.

Our study had some limitations. First, only animal experiments were conducted, and the theories remain unconfirmed clinically. Second, the SHP2/ERK signaling pathway is not the only pathway between elevated CD24 and a good prognosis, and other signaling pathways remain to be further explored. Finally, long-term cognitive behavioral tests after TBI should be further investigated in future studies.

Elevated hippocampal CD24 in astrocytes may promote neural regeneration and protect neurofunctioning, possibly by activating the SHP2/ERK signaling pathway after inducing experimental TBI in mice.

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Disclosure of conflict of interest

None.

Address correspondence to: Chun-Hua Hang and Wei Li, Department of Neurosurgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, #321, Zhongshan Road, Nanjing 210008, China. Tel: +86-25-83106-666-11902; E-mail: hang_neurosurgery@163.com (CHH); Tel: +86-25-83106666-11901; E-mail: lwxzlw@126.com (WL)

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