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Increased CD133$^+$ cell infiltration in the rat brain following fluid percussion injury☆

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Abstract

The prominin-1/CD133 epitope is expressed in undifferentiated cells. Studies have reported that craniocerebral trauma in animal models of fluid percussion injury induces production of a specific stem cell subgroup. It has been hypothesized that fluid percussion injury induces CD133$^+$ cell infiltration in the brain tissue. The present study established a traumatic brain injury model through fluid percussion injury. Immunohistochemical staining showed significantly increased CD133 antigen expression in the rat brain following injury. CD133$^+$ cells were mainly distributed in hippocampal CA1–3 regions, as well as the dentate gyrus and hilus, of the lesioned hemisphere. Occasional cells were also detected in the cortex. In addition, reverse transcription-PCR revealed that no change in CD133 mRNA expression in injured brain tissue. These results suggested that fluid percussion injury induced CD133 antigen expression in the brain tissues as a result of conformational epitope changes, but not transcriptional expression.

Key Words: prominin-1; immunohistochemistry; reverse transcription-PCR; traumatic brain injury; neural regeneration

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INTRODUCTION

Traumatic brain injury (TBI) is the major form of acquired brain injury[1] and is a devastating event that leads to serious long-term disability. Potential self-repair of the cerebrum has been shown, with CD133$^+$ cell populations contributing to neurogenesis and angiogenesis and playing an important role in this reparative mechanism. However, few studies have shown CD133 expression following TBI, and CD133$^+$ cell infiltration in animal brains has not been previously detected.

CD133 is an alternative name for prominin-1, which refers to its epitope. Prominin-1 belongs to a new family of pentaspan membrane glycoproteins and is expressed widely in embryonic mammalian tissues[2-3]. Irrespective of cell types, prominin-1 is specifically associated with microvilli and other plasma membrane protrusions[4-5], and redistribution of prominin-1 in specialized plasma membrane domains plays an important role in stem cell migration. Detailed functions of prominin-1, however, require further elaboration. Growing evidence has shown that CD133$^+$ cells are involved in biological restoration following brain injury. In fact, CD133 antigen expression is restricted to undifferentiated cells, such as endothelial progenitor cells, hematopoietic stem cells, cancer stem cells[6-7], and embryonic epithelium. CD133$^+$ cells are clonogenic cells with multilineage differentiation[8], capable of differentiating into mature endothelial, myotubes[9], neurons, and glial cells. These cells are closely associated with endogenous recovery in the injured brain.

CD133 antigen is expressed in the spinal cord following mild compression injury in rats[10]. In addition, in the fluid percussion injury model, brain injury continuously induces the generation of specific neural stem cell populations. Therefore, it was hypothesized that TBI could induce the generation of CD133$^+$ cells in a rat model. The present study quantified and characterized CD133 antigen expression in a rat TBI model.

RESULTS

Quantitative analysis of experimental animals

A total of 100 rats were randomly assigned to control and injury groups (n = 50 for each), each of which was further divided into five subgroups (n = 10, respectively) for time-point analysis. Injury group rats were subjected to experimental fluid percussion injury, while control group rats underwent anesthesia and surgical procedure without exposure to percussion

injury. Infections were not observed, but five rats died and were excluded from the study. Four rats were selected from each subgroup, respectively, for immunohistochemistry and PCR. Finally, there were 40 rats in control and injury groups, respectively, and 8 rats in each subgroup. A total of 80 rats were included in the final analysis.

CD133 immunohistochemistry in the brain following TBI

Several CD133$^+$ cells were observed in the hippocampus of the control group, but CD133$^+$ cells were not detected in the cortex. However, the number of CD133$^+$ cells increased in the hippocampus at 3 days after TBI; the cells were detected in the CA1–3 regions, dentate gyrus, and hilus (Figures 1A–D; $P < 0.01$). In addition, the number of CD133$^+$ cells significantly increased in the ipsilateral (injured) hippocampus compared with the corresponding region in the contralateral hippocampus ($P < 0.05$). CD133$^+$ cells were also observed in the lesioned ipsilateral cortex and subcortical structures (Figures 1E–H). In contrast, CD133$^+$ cells were not detected in the contralateral cortex (Figures 1e, g). CD133$^+$ endothelial-like cells were also observed around the injured lesion and bilateral hippocampus and subcortical white matter, although few cells were detected in the contralateral cortex.

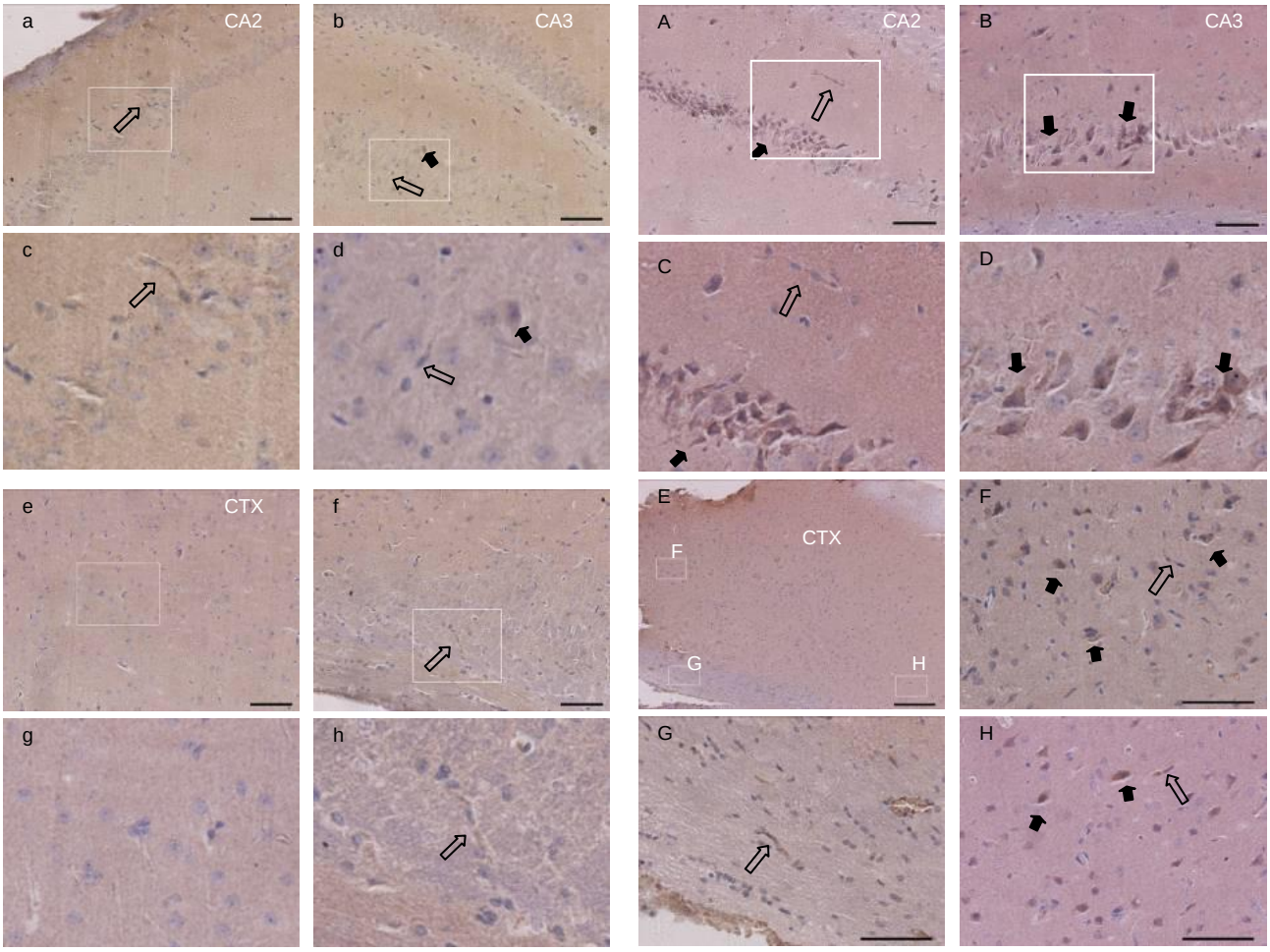

Figure 1 Morphology of CD133$^+$ cells in the rat brain at 7 days (peak) after fluid percussion injury (immunohistochemical staining).

(a–h) Immunoreactivity of CD133 is observed in the contralateral hemisphere following brain injury. In the hippocampus contralateral to injury, weak and scant CD133 expression is observed (a, b). Few CD133$^+$ cells are seen in the contralateral cortex (e), as well as in the subcortical region (f). (c, d, g, h) Higher magnification illustrates boxed areas where CD133$^+$ endothelial-like cells are observed in the hippocampus and cerebral cortex, and subcortical region.

(A–H) CD133 expression is observed in the ipsilateral (injured) hemisphere following brain injury. In the hippocampus, CD133$^+$ cells are clustered in the subgranular zone of CA2 (A) and CA3 regions (B), respectively. (C, D) Higher magnification illustrates boxed areas of A and B. In the cortex (E), expression is observed in the injured cortex (F), subcortical region tissue (G), and relatively far from the injury zone (H).

CD133$^+$ endothelial-and non-endothelial-like cells are marked by open arrows (empty arrows) and black arrows, respectively. CA2, CA3, and pyramidal cells of the subdivisions of Ammon's horn; CTX: cortex. Scale bar is 200 μm in E and 50 mm in the remaining images.

The number of CD133$^+$ cells increased in the ipsilateral (injured) hippocampus in injured animals compared with the control rats at 1 day post-TBI, and expression peaked at 7 days post-TBI ($P < 0.01$). CD133 expression was not detected in lesioned and perilesion cortex at 1 day post-TBI. However, the number of CD133$^+$ cells slightly increased in the ipsilateral cortex at 2 days post-TBI compared with control rats ($P < 0.01$). Expression in these positive structures peaked at 7 days ($P < 0.01$), but decreased at 14 days ($P < 0.01$), post-TBI (Figure 2).

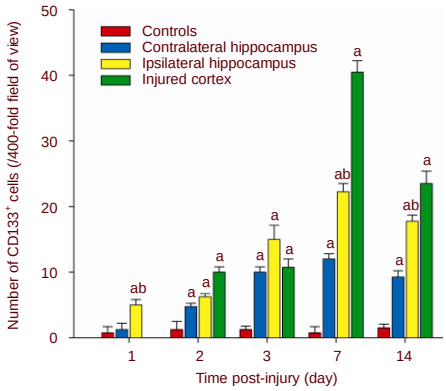

Figure 2 Time course of CD133$^+$ expression in brain tissue following injury.

CD133 expression increases at 1 to 14 days in ipsilateral (injured) hippocampus [two-way analysis of variance, effects of injury ($F = 1\,587.13$, $P < 0.01$), time ($F = 75.16$, $P < 0.01$), and interaction ($F = 72.08$, $P < 0.01$)], and 2 to 14 days in injured cortex [two-way analysis of variance, effects of injury ($F = 2\,489.66$, $P < 0.01$), time ($F = 221.05$, $P < 0.01$), and interaction ($F = 217.93$, $P < 0.01$)] after injury.

The Student *t*-test demonstrates a significant difference in CD133 expression between ipsilateral and contralateral hippocampus at the same time point (F (2, 21) = 16.6, $P < 0.05$).

[a]$P < 0.01$ *vs*. control group; [b]$P < 0.05$ *vs*. contralateral hippocampus. Data are expressed as mean ± SD in four rats from each time point.

Quantification of prominin-1 gene expression in the rat brain following TBI

Temporal gene expression analysis of prominin-1 did not reveal altered gene transcription in brain tissues following TBI, which was similar to protein expression results. A transient slight decrease in mRNA expression was observed at 1 and 2 days post-TBI in the injured hippocampus and cortex compared with control group ($P < 0.05$; Figure 3). There were no significant changes in prominin-1 expression in the cortex and hippocampus at 7 and 14 days post-TBI.

DISCUSSION

The present study showed prominin-1 protein expression in normal and fluid percussion injury brains for the first time. Although CD133 expression was extremely minimal in control brains, protein expression increased in injured brain tissues and reached peak values by 7 days after TBI. Increased CD133 antigen expression following TBI was consistent previous results[11], which showed CD133 immunogold particles in the cerebral cortex proximal to surgical injury using electron microscopic immunocytochemical techniques. However, the pathological mechanisms of surgical injury are different than TBI, and the present study did not thoroughly identify temporal and spatial patterns of CD133 antigen expression. Rats in the present study were subjected to fluid percussion brain injury, which is a classic model for TBI. In contrast to the previous study, CD133 antigen was wildly distributed in the cortex and hippocampus, and expression was detected on multiple cellular profiles.

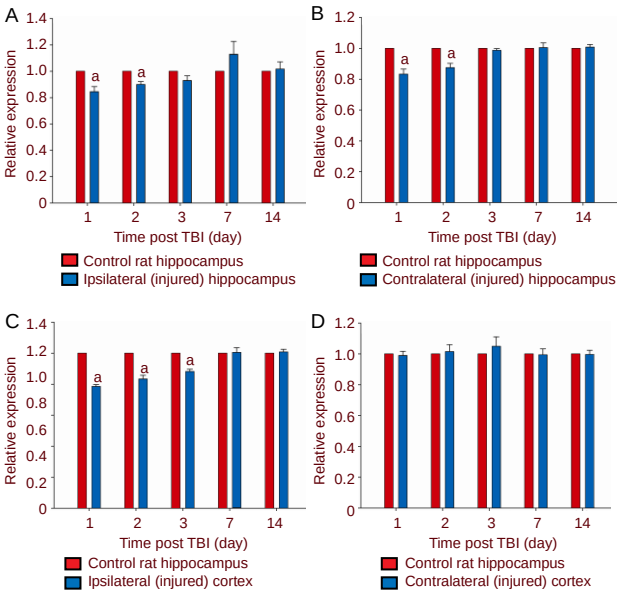

Figure 3 Quantitative PCR analysis of prominin-1 mRNA expression following traumatic brain injury (TBI).

(A) Prominin-1 gene expression in ipsilateral (injured) hippocampus.

(B) Prominin-1 gene expression in contralateral hippocampus.

(C) Prominin-1 gene expression in ipsilateral cortex.

(D) Prominin-1 gene expression in contralateral cortex.

Average mRNA expression in control brains is set to 1. [a]$P < 0.05$ *vs*. control rat tissue. Values are expressed as mean ± SD from four rats in each group. Two-way analysis of variance and least significant difference *t*-test were used.

Specifically, CD133$^+$ cells were identified in the subgranular zone of the dentate gyrus, the CA3 pyramidal cell region, the dentate gyrus hilus, the corpus callosum, and the ipsilateral cortex. CD133$^+$ endothelial-like cells were also detected in the injured region boundary, as well as the contralateral and ipsilateral (injured) hippocampus. Endothelial-like cells are likely committed cells that are between the immature and terminally differentiated endothelial cell stage;

CD133 expression is down-regulated when these cells differentiate into mature endothelial cells. These results suggested that $CD133^+$ cells in injured tissues have the potential for multilineage differentiation. Indeed, $CD133^+$ cells have been reported to proliferate, as well as differentiate into mature CNS cells[12-13] and endothelial cells[6], which is regarded as the predominant repair mechanism in the adult central nervous system following TBI[14].

Interestingly, although CD133 antigen expression was extensively distributed in the injured tissues, CD133 gene expression did not increase following TBI. As previously noted, prominin-1 mRNA is widely distributed in adult tissues, but CD133 expression has been primarily detected in human embryonic tissues. In adult tissues, CD133 expression has been shown in the small intestine[15], eye[16], epididymis, and testis[4]. CD133 protein undergoes heavy modification of its eight different N-linked glycosylation sites by glycosylation, and cell maturation is associated with rapid down-regulation of CD133 glycosylation. Treatment with antibody against CD133 recognizes the glycosylated epitope, and increased CD133 antigen expression is likely attributed to altered antigen epitopes, rather than up-regulation of gene transcription[17]. CD133 is typically used as a marker for progenitor/stem cells[18], and increased CD133 protein expression around injured brain tissues suggests accumulation of stem cells. Results from the present study were consistent with the notion that TBI activates proliferation, migration, and differentiation of stem/progenitor cells[19]. Increased numbers of $CD133^+$ cells in injured tissue could arise from migrating stem/progenitor cells, such as subventricular zone progenitor cells or bone marrow-derived stem cells that circulate within the blood, or dedifferentiation of mature neuroglia could induce up-regulation of CD133 epitope expression[20].

In summary, TBI increased the number of $CD133^+$ cells in the rat brain. Although the role of CD133 in reparative mechanisms following TBI was not identified in the present study, recent evidence indicates that $CD133^+$ cell therapy could provide a novel therapeutic strategy for modifying tissue in the injured spinal cord and infarcted myocardium[21-22]. $CD133^+$ cells could offer promising opportunities for the treatment of TBI. However, future studies should focus on the source and function of $CD133^+$ cells in the brain following TBI.

MATERIALS AND METHODS

Design

A randomized, controlled, animal study.

Time and setting

The experiments were performed at the Laboratory of Tianjin Neurological Institute, Tianjin, China from November 2009 to January 2011.

Materials

A total of 100 adult, male, Wistar rats, weighing 300–320 g, were purchased from Vital River Laboratories, Beijing, China (license No. SCXK (Jing) 2006-0009). The rats were individually housed under a 12-hour light/dark cycle with regular food and water supply. Animal housing and care were maintained according to the *Guidance Suggestions for the Care and Use of Laboratory Animals*, published by the Ministry of Science and Technology of China[23].

Methods

Establishment of fluid percussion injury models

A fluid percussion injury device (Model 01-B, New Sun, Health Products, Cedar Bluff, VA) was used to induce brain injury[24]. Briefly, rats were anesthetized with 10% chloraldurat (3 mL/kg) and placed on a stereotaxic frame. Following aseptic surgical procedures, a midline incision was made, soft tissues were removed, and a 4.0-mm craniotomy was performed over the right parietal skull to expose the dura (4.0 mm posterior from bregma and 3.0 mm lateral to the sagittal suture). A Luer-lok connector (2.6 mm inner diameter) was cemented to the skull with cranioplastic cement. A syringe filled with sterile saline was inserted into the Luer-lok syringe fitting and connected to the fluid percussion device. Pressure pulse (182.38–202.65 kPa) and pulse duration were measured by a transducer attached to the fluid percussion device. Following injury, the incision was suture-closed and the rats were allowed to recover from anesthesia. Control rats underwent a craniotomy, but did not experience percussion injury.

CD133 immunohistochemistry in the rat brain following TBI

The rats were transcardially perfused with normal saline followed by 4% buffered paraformaldehyde. The damaged brain regions were transversally cut into 2-mm thick sections and embedded in paraffin. Slices (4-μm thick) were cut and mounted onto glass slides. The slides were then boiled at 95–100°C in citrate buffer for antigen retrieval. The slides were then treated with 1.5% normal horse serum for 30 minutes, followed by incubation overnight at 4°C with polyclonal rabbit anti-rat CD133 (1: 200; Abcam, Cambridge, MA, USA). After incubation with biotinylated goat anti-rabbit IgG for 1 hour (1: 100; Vector Laboratories, Burlingame, CA, USA), the sections were incubated with ABC reagent (Vector Laboratories, Burlingame, CA, USA) for 30 minutes, followed by 0.015% hydrogen peroxide and 0.05% diaminobenzidine for 5–10 minutes. The number of $CD133^+$ cells in each section was quantified (view 400 ×, Leica, Wetzlar, Germany) in five fields of view in a blinded fashion, and the average number of positive cells per field of view was obtained[25].

Quantitative real-time PCR assays of prominin-1 gene expression in the rat brain following TBI

Total RNA was isolated from brain tissues using TRIZOL® Reagent (Invitrogen, Carlsbad, CA, USA) and DNase treatment according to manufacture instructions. For real-time PCR, reverse transcription of RNA

samples was performed using PrimeScript™ RT reagent (Takara Bio, Shiga, Japan). The relative amount of target mRNA was determined using SYBR® Premix Ex Taq™ II (Takara Bio) according to manufacture instructions. PCR primers used to detect prominin-1 were as follows: sense strand: 5'- AAC GTG GTC CAG CCG AAT G -3'; antisense strand: 5'- CCC AGG ATG ACG CAG ATA AGA AC -3'. Cycle thresholds for single reactions were determined using MyiQ software (Bio-Rad, Hercules, CA, USA), and target genes were normalized against GAPDH expression. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative changes in gene expression.

Statistical analysis

Data were expressed as mean ± SD and were analyzed by analysis of variance for multiple comparisons of western blot and PCR data using SPSS 13.0 software (SPSS, Chicago, IL, USA). Two-way analysis of variance with *post hoc* LSD-*t* test was used to compare data difference among groups. $P < 0.05$ was considered statistically significant.

Author contributions: Jianning Zhang was responsible for funding and authorized the study. Shenghui Li, Ziwei Zhou, Dashi Zhi, and Ming Wei conducted the experiments, conceived and designed the study, and wrote a draft of the manuscript. Chengwei Jing conducted animal experiments, conceived and designed this study, and drafted the manuscript.

Conflicts of interest: None declared.

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Ethical approval: This study received permission from the Animal Care and Ethics Committee of Tianjin Medical University.

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