Brain repair by hematopoietic growth factors in the subacute phase of traumatic brain injury

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OBJECTIVE Traumatic brain injury (TBI) is a major cause of long-term disability and death in young adults. The lack of pharmaceutical therapy for post-acute TBI recovery remains a crucial medical challenge. Stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF), which are 2 key hematopoietic growth factors, have shown neuroprotective and neurorestorative effects in experimental stroke. The objective of this study was to determine the therapeutic efficacy of combined treatment (SCF + G-CSF) in subacute TBI.

METHODS Young-adult male C57BL mice were subject to TBI in the cortex of the right hemisphere. After TBI induction, mice were randomly divided into 2 groups: a vehicle control group and an SCF + G-CSF treatment group. Mice without TBI served as sham operative controls. Treatment was initiated 2 weeks after TBI induction. SCF (200 µg/kg) and G-CSF (50 µg/kg) or an equal volume of vehicle solution was subcutaneously injected daily for 7 days. A battery of neurobehavioral tests for evaluation of memory and cognitive function (water maze and novel object recognition tests), anxiety (elevated plus maze test), and motor function (Rota-Rod test) was performed during the period of 2–9 weeks after treatment. Neurodegeneration and dendritic density in both hemispheres were determined through histochemistry and immunohistochemistry at 11 weeks posttreatment.

RESULTS Water maze testing showed that TBI-impaired spatial learning and memory was restored by SCF + G-CSF treatment. The findings from the elevated plus maze tests revealed that SCF + G-CSF treatment recovered TBI-caused anxiety and risk-taking behavior. There were no significant differences between the treated and nontreated TBI mice in both the Rota-Rod test and novel object recognition test. In the brain sections, the authors observed that widespread degenerating neurons were significantly increased in both hemispheres in the TBI-vehicle control mice. TBI-induced increases in neurodegeneration were significantly reduced by SCF + G-CSF treatment in the contralateral hemisphere, making it no different from that of the sham controls. Dendritic density in the frontal cortex of the contralateral hemisphere was significantly reduced in the TBI-vehicle control mice, whereas SCF + G-CSF-treated TBI mice showed significant increases of the dendritic density in the same brain region. SCF + G-CSF-treated TBI mice also showed a trend toward increasing dendritic density in the contralateral hippocampus.

CONCLUSIONS SCF + G-CSF treatment in the subacute phase of TBI restored TBI-impaired spatial learning and memory, prevented posttraumatic anxiety and risk-taking behavior, inhibited TBI-induced neurodegeneration, and enhanced neural network remodeling. These findings suggest the therapeutic potential of hematopoietic growth factors for brain repair in the subacute phase of TBI.

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KEY WORDS traumatic brain injury; hematopoietic growth factor; SCF; G-CSF; brain repair; functional recovery; stem cell factor; granulocyte colony-stimulating factor

TRAUMATIC brain injury (TBI) is a sudden injury to the brain caused by external forces such as falls, motor vehicle collisions, violence, and sports. TBI represents a serious medical problem that results in a heavy health care burden worldwide.^{23,32} TBI is a major cause of death and disability, especially in children and

young adults.^{3,32} In 2013, more than 2.8 million people in the United States suffered a TBI, and more than 56,000 Americans died as a result of TBI.³² The total lifetime cost for TBI has been estimated at approximately \$406 billion in the United States.⁴

Post-TBI recovery consists of 3 phases: acute, subacute,

ABBREVIATIONS CCI = controlled cortical impact; G-CSF = granulocyte colony-stimulating factor; MAP2 = microtubule-associated protein 2; rpm = rotation per minute; SCF = stem cell factor; TBI = traumatic brain injury.

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and chronic. The exact duration of each phase is different for individuals according to their age, the injury location, and severity of TBI. In general, the acute phase is the first 24 hours after TBI, the subacute phase is the time period between 1 day and 3 weeks post-TBI, and the chronic phase begins 3 weeks after TBI.^{18,30,33,34}

Clinical manifestations include motor deficits, cognitive impairment, and emotional or behavioral disorders. Current treatment for TBI includes medical and surgical interventions to relieve intracranial mass effect and pressure in the acute and subacute phases. Physical therapy is the only current treatment in the subacute and chronic phases of TBI. Developing a pharmaceutical therapy for TBI recovery has been pointed out as a critical need and current gap in TBI research.¹⁰ Until now, effective pharmacological therapy that can prevent secondary neuron injury and improve TBI recovery in the postacute phases of TBI has not existed.^{10,31}

Advances in understanding of the neurobiology of 2 essential hematopoietic growth factors, stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF), suggest that they may play a role in reducing brain damage and enhancing brain repair after brain injury. In addition to their primary effects in regulating hematopoiesis,^{35,40,41} SCF and G-CSF have been demonstrated to play roles in the CNS. Receptors for SCF and G-CSF are expressed in neurons^{25,39} and cerebral endothelial cells,³⁷ and both SCF and G-CSF can pass through the blood-brain barrier.^{25,37} SCF and G-CSF show neuroprotection in vitro.^{9,25} Administration of SCF³⁹ or G-CSF^{12,16,17,25–28,39} in the acute phase of cerebral ischemia leads to reduction of infarction size.

In addition to their neuroprotective effects, SCF and G-CSF combination treatment (SCF + G-CSF) enhances brain repair and improves functional recovery when administered in the subacute or chronic phase in animal models of stroke.^{14,36} Injections of SCF + G-CSF during the chronic phase of experimental stroke also increase neuronal network rewiring.^{5–7,38} However, it remains to be determined whether SCF + G-CSF can enhance brain repair in TBI. The purpose of this study was to demonstrate the therapeutic efficacy of SCF + G-CSF treatment in the subacute phase of TBI.

Methods

All procedures of animal experiments were approved by the Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Controlled Cortical Impact Model of TBI

A controlled cortical impact (CCI) model of TBI was used in this study to make a reproducible and consistent severe TBI model in young-adult mice through a controlled impactor (Impact One Stereotaxic Impactor, Leica Biosystems). Nineteen C57BL mice (male, 12 weeks old) were anesthetized with Avertin (0.4 g/kg body weight, intraperitoneal) and then immobilized in a stereotaxic frame. An incision was made in the midline on the head, and a

4-mm-diameter circular craniotomy on the right side was performed with a dental burr drill, taking care to avoid thermal injury by using irrigation. This opening was centered at the following coordinates: 0.0 mm to the bregma and 2.0 mm lateral to the midline (this area is the somatosensorimotor cortex of the forelimb and hindlimb in mice).

After the dural surface was exposed, the tip of the impactor was carefully positioned at the center of the craniotomy and then angled 4° medially. A 3-mm flat tip with a strike velocity of 1.5 m/sec, a 2-mm strike depth from the surface of the dura, and an 8.5-sec contact time was used to induce cortical TBI. The incision was closed with interrupted 4-0 nylon surgical sutures. Sham animals were handled in the same manner, except the bone flap of the craniotomy was left in place and the cortex was not disrupted.

Groups and Treatment

After induction of TBI, mice were randomly divided into 2 groups: a vehicle control group (n = 9) and an SCF + G-CSF treatment group (n = 10). Mice without TBI served as sham operative controls (n = 5). Treatment was started 2 weeks after TBI induction, corresponding to the subacute phase of TBI. Recombinant mouse SCF (200 µg/kg/day) (PeproTech), recombinant human G-CSF (50 µg/kg/day) (Amgen), or an equal volume of vehicle solution was subcutaneously injected daily for 7 days. Previous work in our laboratory has shown that combination therapy (SCF + G-CSF) for 1 week at the doses delivered results in the best brain repair outcome in the animal model of stroke.^{22,36}

After treatment, several neurobehavioral tests were performed to evaluate functional recovery. Elevated plus maze, novel object recognition, water maze, and Rota-Rod tests were performed 2, 3, 4, or 9 weeks after treatment, respectively. Two weeks after the final Rota-Rod tests, mice were killed for histological analysis (Fig. 1).

Neurobehavioral Tests

A battery of neurobehavioral tests (including the elevated plus maze, novel object recognition, water maze, and Rota-Rod) was used in this study. During these tests, animal behavior was captured using an ANY-Maze Video Tracking System (Stoelting Co.).

Elevated Plus Maze

The elevated plus maze test assesses posttraumatic anxiety and risk-taking behavior. The apparatus of the elevated plus maze comprised a “plus” or cross-shaped maze elevated above the floor with 2 enclosed arms (protecting), 2 open arms (stressful), and a center area. Mice were placed in the center of the apparatus by facing the closed arms and were allowed to explore the maze freely for 5 minutes. The total time spent in the open arms or closed arms was recorded.

Novel Object Recognition Test

The novel object recognition test evaluates cognition, particularly recognition memory. The spontaneous tendency of mice to spend time exploring a novel object or a familiar one was examined. The baseline levels of lo-

FIG. 1. Schematic flow chart of experimental timeline. Two weeks after TBI induction, mice were randomized to receive subcutaneous (s.c.) injections of SCF + G-CSF or an equal volume of vehicle solution for 7 days. Neurobehavioral tests were performed during the period of 2–9 weeks post-TBI. Eleven weeks after treatment, mice were killed to determine neurodegeneration and neural network density. w = week.

comotor activity were recorded during the familiarization trials (trials 1 and 2). Two identical objects (trial 3) or a familiar object and a new object (trial 4) were placed in the testing container to evaluate recognition memory.

Water Maze Test

The water maze test examines spatial learning and memory. A 4-foot-diameter water tank was filled with water (26°C, room temperature) and mixed with nontoxic white paint. A circular platform was hidden 1 cm beneath the surface of the water at a fixed position in the center of 1 of 4 imaginary quadrants. A water maze test was performed daily for a total of 5 days. Each daily trial consisted of four 1-minute swimming trials starting randomly from each of 4 starting positions.

Rota-Rod Test

The Rota-Rod test examines motor function in mice. Mice were placed on the Rota-Rod (Coulbourn Instruments), facing away from the experimenter. Each mouse was placed on the rod at 0 rotation per minute (rpm). The rod was then accelerated steadily from 2 rpm to 20 rpm during a 5-minute testing period (by 2 rpm every 30 seconds). The latency to fall on the floor plate was recorded (maximum 5 minutes).

Histology and Immunohistochemistry

Fluoro-Jade C (Millipore) staining identifies neuronal degeneration.²⁴ Brain sections were mounted on Superfrost Plus Slides (Fisher Scientific) and allowed to dry overnight at room temperature. The slides were immersed in 100% ethanol and 70% ethanol. After rinsing in distilled water, the slides were immersed in 0.06% potassium permanganate (KMnO₄) (Sigma). Brain sections were then rinsed in distilled water, incubated with 0.001% Fluoro-Jade C for 30 minutes, dried at room temperature overnight in the dark, cleared by brief immersion in xylene, and mounted with DePeX mounting media (Electron Microscopy Sciences). Fluoro-Jade C–positive staining was captured with a 20× objective using a fluorescence microscope (Zeiss Axio Imager A1) and analyzed by ImageJ software.

Microtubule-associated protein 2 (MAP2) is a neuron-specific cytoskeletal protein that is enriched in dendrites. In this study, neuronal dendrites were specifically identified with an antibody against MAP2 through free-floating

immunofluorescent staining. Brain sections were washed with phosphate-buffered saline (pH 7.4) (Boston BioProducts). Nonspecific binding was blocked with 10% normal goat serum diluted by 1% bovine serum albumin (Sigma) and 0.25% Triton X-100 (Sigma) for 1 hour at room temperature. Brain sections were incubated with the primary antibody, MAP-2 (1:500) (Sigma), overnight at 4°C. After rinsing with phosphate-buffered saline, brain sections were incubated with the secondary antibody, DyLight 488-goat anti-mouse IgG (1:200) (Vector Laboratories), for 2 hours at room temperature in the dark. Free-floating brain sections were mounted on Superfrost Plus Slides with VECTASHIELD HardSet Antifade Mounting Medium (Vector Laboratories). MAP2-immunopositive staining was captured with a fluorescence microscope (Zeiss Axio Imager A1) through a 40× objective. The density of MAP2-labeled dendrites was analyzed by ImageJ software.

Statistical Analysis

One-way or 2-way ANOVA with Bonferroni or Tukey correction for multiple comparisons (GraphPad Prism software) was used for data analysis. A $p < 0.05$ was considered statistically significant. Data are presented as the mean $\pm$ SEM.

Results

SCF + G-CSF Treatment in the Subacute Phase of Brain Trauma Improves Neurobehavioral Outcomes

Taking longer to find the hidden platform in the water maze test indicates impairment in spatial learning and memory. During water maze testing, there were no significant differences in escape latency among the experimental groups of sham control, TBI-vehicle control, and TBI-SCF + G-CSF treatment at days 1 and 2 ($p > 0.05$). However, TBI-vehicle control mice spent significantly longer times to find the hidden platform at days 3, 4, and 5 compared with the sham operative control mice (sham vs TBI-vehicle: $p < 0.05$ at day 3, $p < 0.001$ at day 4, $p < 0.001$ at day 5). This observation indicates that TBI causes impairments in spatial learning and memory. In contrast, mice treated with SCF + G-CSF took significantly shorter times to find the hidden platform at days 3, 4, and 5 than the TBI-vehicle control mice (TBI-vehicle vs TBI-SCF + G-CSF: $p < 0.05$ at day 3, $p < 0.01$ at day 4, and $p < 0.05$ at

day 5). Importantly, the escape latency of SCF + G-CSF-treated TBI mice at days 3, 4, and 5 was not different from that of the sham controls ($p > 0.05$). These findings suggest that SCF + G-CSF treatment in the subacute phase of TBI restores TBI-induced deficits in spatial learning and memory (Fig. 2).

The elevated plus maze test has been used to evaluate posttraumatic anxiety and risk-taking behaviors.²¹ Increased time in the open arms indicates anxiety and risk-taking behaviors. We observed that TBI-vehicle control mice stayed in the open arms significantly longer than in the closed arms ($p < 0.05$), suggesting that our TBI model causes anxiety and risk-taking behaviors. However, the SCF + G-CSF-treated TBI mice showed a pattern similar to that of the sham controls (that is, no differences were found between the time spent in the open arms and the closed arms) (Fig. 3), indicating that SCF + G-CSF treatment repairs the TBI-induced anxiety and risk-taking behaviors.

The novel object recognition test examines recognition memory by assessing for interactions with novel objects and evaluates nonspatial memory in an open field. We observed that exploration time spent on the novel or familiar objects was not statistically different across experimental groups. The SCF + G-CSF-treated TBI mice showed a trend toward increased exploration of the familiar objects ($p = 0.13$) (Fig. 4). This finding may be related to the treatment-induced recognition memory.

Shortened latency to fall from a rotating rod in the Rota-Rod test demonstrates motor impairment. We found that the latency to fall from the rotating rod was significantly decreased in the TBI-vehicle control mice compared with the sham controls, indicating impaired motor function in the TBI mice. The latency to fall from the rotating rod in the SCF + G-CSF-treated TBI mice did not show significant differences compared with that of the TBI-vehicle control mice, whereas a significantly decreased latency to fall was seen between SCF + G-CSF-treated TBI mice and the sham control mice (Fig. 5). This observation indicates that the TBI-induced impairments in motor function are not improved by SCF and G-CSF treatment.

SCF + G-CSF Treatment in the Subacute Phase of Brain Trauma Inhibits Neurodegeneration in the Brain

Fluoro-Jade C staining is used to identify degenerating neurons. In the contralateral hemisphere, the Fluoro-Jade C-positive degenerating neurons were significantly increased in the TBI-vehicle control mice compared with those in sham controls ($p < 0.05$), indicating that severe injury to the cortex of the right hemisphere causes neurodegeneration in the contralateral left hemisphere > 3 months after TBI induction. In contrast, SCF + G-CSF treatment led to a significant reduction in Fluoro-Jade C-labeled degenerating neurons in the contralateral hemisphere compared with the TBI-vehicle control ($p < 0.01$). The Fluoro-Jade C-positive degenerating neurons in the brains of SCF + G-CSF-treated mice were not different from those of the sham control mice ($p > 0.05$) (Fig. 6A–C). This observation suggests that SCF + G-CSF intervention in the subacute phase of TBI prevents neurodegeneration in the contralateral hemisphere.

FIG. 2. Graph of water maze test results shows that SCF + G-CSF treatment improved TBI-impaired spatial learning and memory. No significant differences among experimental groups were seen at day 1 and day 2 testing. During day 3, day 4, and day 5 testing, the latency to find the hidden platform (escape latency) was significantly increased in the TBI-vehicle control mice compared with sham controls (* $p < 0.05$, *** $p < 0.001$), whereas SCF + G-CSF-treated TBI mice showed a significant reduction in the escape latency compared with the TBI-vehicle control mice (# $p < 0.05$, ## $p < 0.01$). There was no significant difference between SCF + G-CSF-treated TBI mice and sham controls during the water maze testing. Mean $\pm$ SEM. G = G-CSF; S = SCF. Figure is available in color online only.

In the ipsilateral hemisphere, the number of Fluoro-Jade C-positive degenerating neurons in the brains of TBI-vehicle control mice was significantly greater than that in sham control mice ($p < 0.05$). SCF + G-CSF treatment did not reduce the Fluoro-Jade C-positive cells in the ipsilateral hemisphere (Fig. 6A and D).

FIG. 3. Graph of elevated plus maze test results shows that SCF + G-CSF treatment repaired TBI-induced anxiety and risk-taking behaviors. Note that TBI-vehicle control mice spent significantly longer time in the open arms than in the closed arms. The time spent exploring in the open arms and the closed arms was not different between the sham control mice and SCF + G-CSF-treated TBI mice. * $p < 0.05$. Mean $\pm$ SEM. G = G-CSF; S = SCF. Figure is available in color online only.

FIG. 4. Graph shows that novel object recognition was not significantly enhanced by the treatment in TBI mice. Note that SCF + G-CSF–treated TBI mice showed a trend toward increased exploration time on the familiar (old) objects. Mean $\pm$ SEM. G = G-CSF; S = SCF. Figure is available in color online only.

SCF + G-CSF Treatment in the Subacute Phase of Brain Trauma Increases Dendritic Density in the Frontal Cortex of the Contralateral Hemisphere

MAP2 is a signature protein for identifying neuronal dendrites. In the frontal cortex of the contralateral hemisphere, TBI-vehicle control mice showed a trend toward decreasing MAP2-positive dendrites compared with the sham controls ($p = 0.10$). SCF + G-CSF treatment significantly increased the MAP2-positive dendrites in the contralateral frontal cortex compared with the TBI-vehicle controls ($p < 0.05$). The SCF + G-CSF–increased density of MAP2-positive dendrites was not different from that of the sham controls (Fig. 7A and B).

In the cortex next to the TBI cavity, the TBI-vehicle control mice displayed a trend toward decreased MAP2-labeled dendrites compared with the sham controls ($p = 0.12$). No differences were observed between the TBI-vehicle controls and SCF + G-CSF–treated mice (Fig. 7A and C).

In the hippocampal CA3, SCF + G-CSF–treated mice showed a trend toward an increased density of MAP2-labeled dendrites in the contralateral hemisphere ($p = 0.14$) but not in the ipsilateral CA3 (Fig. 7D).

These findings suggest that SCF + G-CSF treatment in the subacute phase of TBI prevents dendritic reduction in the contralateral hemisphere.

Discussion

We believe that the CCI model used in this study accurately reproduces many of the cognitive, motor, and neurobehavioral deficits observed in severe human TBI. To our knowledge, the current study provides the first evidence supporting the therapeutic efficacy of SCF + G-CSF treatment on enhancing TBI recovery in the subacute phase of TBI. Specifically, TBI-impaired spatial learning and memory was improved, there was lessened post-traumatic anxiety and risk-taking behavior, TBI-induced widespread neurodegeneration was reduced, and neural network remodeling was increased.

FIG. 5. Graph shows that TBI-induced impairments in motor function were not improved by SCF + G-CSF treatment. Note that there was no significant difference in the latency to fall from the rotating rod between TBI-vehicle control mice and SCF + G-CSF–treated TBI mice. Both TBI-vehicle controls and SCF + G-CSF–treated TBI mice showed significant reductions in the latency to fall from the rotating rod compared with sham controls (sham vs TBI-vehicle: $**p < 0.01$, $****p < 0.0001$; sham vs TBI-SCF + G-CSF: $###p < 0.01$, $####p < 0.0001$). Mean $\pm$ SEM. Figure is available in color online only.

In this study, the TBI-induced deficits in motor function, however, were not repaired by the SCF + G-CSF treatment. In our previous work,^{5,7} SCF + G-CSF treatment in the chronic phase of experimental stroke resulted in significant motor improvement during the Rota-Rod test. The mouse model of experimental stroke causes cortical infarct in the somatosensory motor cortex of the right hemisphere, whereas other brain regions are not directly damaged by this model. In contrast, the TBI model used in the current study led not only to severe direct damage in the motor cortex of the right hemisphere, but also to diffuse injury across the brain, which was evidenced by widespread neurodegeneration in both hemispheres (Fig. 6).

Yet it remains to be determined whether increasing the dosage or duration of the SCF + G-CSF treatment in the subacute phase would improve motor function after a severe TBI and which phase of a severe TBI is the optimal time for SCF + G-CSF intervention to obtain maximal recovery. It is also possible that the severity of the contusion seen after our CCI injury led to irreversible damage to the motor neurons. Future experiments using a less severe brain injury model may address the issue of motor recovery using SCF + G-CSF.

Others have also shown that moderate to severe TBI causes persistent and progressive neurodegeneration.^{8,19} Using a similar CCI model, Loane et al.¹⁹ observed progressively increased neuron loss at 5, 12, and 52 weeks post-TBI induction. In the current study, 14 weeks after the induction of TBI, we found significantly increased degenerating neurons in both hemispheres. These studies demonstrate that progressive and long-lasting neurodegeneration occurs in the brain after TBI.

Daily injections for 7 days of SCF + G-CSF in the subacute phase (2 weeks post-TBI) prevented TBI-induced neurodegeneration in the contralateral hemisphere, but

FIG. 6. SCF + G-CSF treatment inhibited TBI-induced neurodegeneration. **A:** Representative images showing Fluoro-Jade C-positive degenerating neurons in both the contralateral and ipsilateral hemispheres. **B:** Schematic diagram indicating the imaged areas in both hemispheres. **C:** Quantification data of Fluoro-Jade C-positive degenerating neurons in the contralateral hemisphere. **D:** Quantification data of Fluoro-Jade C-positive degenerating neurons in the ipsilateral hemisphere. Scale bars = 50 μ m. * $p < 0.05$, ** $p < 0.01$. Mean $\pm$ SEM. G = G-CSF; S = SCF. Figure is available in color online only.

had no effect on degenerating neurons in the ipsilateral hemisphere. Whether an enhanced treatment paradigm (that is, increased dosage and/or treatment duration) would also inhibit neurodegeneration in the severely damaged ipsilateral hemisphere is an open question to be addressed in future studies.

The mechanism underlying the SCF + G-CSF-inhibited neurodegeneration in the subacute phase of TBI remains unclear. Numerous studies have demonstrated neuroprotective efficacy of SCF and G-CSF in different conditions. SCF acts as a neurotrophic factor supporting neuron survival during development of the peripheral nervous system.^{2,13} SCF⁹ or G-CSF²⁵ protects cultured neurons from programmed neuron death through the PI3K pathway. SCF,³⁹ G-CSF,^{12,16,25–28,39} or SCF + G-CSF³⁹ treatment in the acute phase of stroke reduces infarction size in animal models. Importantly, infarction size is also reduced when G-CSF¹⁷ or SCF + G-CSF¹⁴ treatment is given in the subacute phase of stroke.

Emerging evidence shows that the integrity of brain

networks plays a key role in functional recovery after brain injury. Recent advances in studying brain function have shown that the frontal cortex, which gathers connections with many brain regions, including multiple memory systems such as the hippocampus, is responsible for processing abstract thought, learning, memory, and executive function.^{1,11} Clinical imaging studies have revealed that TBI survivors who have problems with learning and memory, cognitive function, decision making, and risk-taking behavior show impaired brain networks across the brain, especially in the frontal cortex, that manipulate high-level cognitive and executive function.^{15,20}

In the present study, SCF + G-CSF treatment in the subacute phase prevented TBI-reduced dendritic density in the frontal cortex of the contralateral hemisphere. Interestingly, TBI-induced neurodegeneration in the contralateral hemisphere (including the frontal cortex) was also prevented by the SCF + G-CSF treatment. Both of these positive results of SCF + G-CSF treatment may play a key role in maintaining the integrity of the brain networks that are

FIG. 7. SCF + G-CSF treatment increased dendritic density in the contralateral hemisphere after TBI. **A:** Representative images showing MAP2-labeled dendrites in the cortex of both hemispheres. Images shown in the contralateral cortex were obtained in the frontal cortex, which is the homotopic area to the TBI cavity. Images shown in the ipsilateral cortex were obtained in the cortex adjacent to the TBI cavity. Scale bars = 25 μ m. **B and C:** Quantification data of dendritic density in the bilateral cortex. Note that MAP2-labeled dendrites in the frontal cortex of the contralateral hemisphere were significantly increased in SCF + G-CSF-treated TBI mice compared with TBI-vehicle controls. No differences were found between TBI-vehicle control mice and SCF + G-CSF-treated TBI mice in the ipsilateral cortex. **D:** Quantification data of dendritic density in the CA3 subregion of the hippocampus. Note that a trend toward increased dendritic density is shown in the contralateral CA3 of SCF + G-CSF-treated TBI mice. * $p < 0.05$. Mean $\pm$ SEM. G = G-CSF; S = SCF. Figure is available in color online only.

important for high-level management of cognitive and executive function. The effectiveness of SCF + G-CSF treatment on maintaining the integrity of brain networks may be necessary for restoring TBI-impaired spatial learning and memory and for preventing posttraumatic anxiety and risk-taking behavior.

There may also be a direct effect of SCF + G-CSF on promoting dendritic growth that results in the increased contralateral frontal cortex dendritic density. Our previous study demonstrated that SCF + G-CSF can directly promote neuronal process outgrowth and neuronal connection formation through the regulation of PI3K/AKT/NF- κ B/BDNF (brain-derived neurotrophic factor).²⁹ SCF + G-CSF treatment in the chronic phase of experimental stroke increases dendritic branching and synaptic generation in the ipsilateral somatosensory and motor cortex.^{5,6} If the SCF + G-CSF-enhanced neural network remodeling in the ipsilateral somatosensory and motor cortex is blocked, motor function recovery is eliminated.^{5,7} Although the stroke studies have demonstrated a causal link between SCF + G-

CSF-enhanced neural network remodeling and functional improvement, the same mechanism after TBI remains to be determined.

Conclusions

SCF + G-CSF treatment in the subacute phase of TBI restores TBI-impaired spatial learning and memory, prevents posttraumatic anxiety and risk-taking behavior, inhibits TBI-induced neurodegeneration, and enhances neural network remodeling. These findings suggest the therapeutic potential of hematopoietic growth factors for brain repair in the subacute phase of TBI.

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Disclosures

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Author Contributions

Conception and design: Zhao. Acquisition of data: Toshkezi, Kyle, Longo. Analysis and interpretation of data: Zhao. Drafting the article: Zhao, Toshkezi. Critically revising the article: Zhao. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Zhao. Statistical analysis: Kyle, Longo. Administrative/technical/material support: Chin. Study supervision: Zhao, Chin.

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