Suppression of neurocan and enhancement of axonal density in rats after treatment of traumatic brain injury with scaffolds impregnated with bone marrow stromal cells

Laboratory investigation

ASIM MAHMOOD, M.D.,† HONGTAO WU, M.D.,† CHANGSHENG QU, M.D.,† SELINA MAHMOOD, B.Sc.,† YE XIONG, M.D., Ph.D.,† DAVID L. KAPLAN, Ph.D.,‡ AND MICHAEL CHOPP, Ph.D.†,‡

Departments of †Neurosurgery and ‡Neurology, Henry Ford Hospital, Detroit; †Department of Physics, Oakland University, Rochester, Michigan; and ‡Department of Biomedical Engineering, Tufts University, Boston, Massachusetts

Object. Neurocan is a major form of growth-inhibitory molecule (growth-IM) that suppresses axonal regeneration after neural injury. Bone marrow stromal cells (MSCs) have been shown to inhibit neurocan expression in vitro and in animal models of cerebral ischemia. Therefore, the present study was designed to investigate the effects of treatment of MSCs impregnated with collagen scaffolds on neurocan expression after traumatic brain injury (TBI).

Methods. Adult male Wistar rats were injured with controlled cortical impact and treated with saline, human MSCs (hMSCs) (3 × 10⁶) alone, or hMSCs (3 × 10⁶) impregnated into collagen scaffolds (scaffold + hMSCs) transplanted into the lesion cavity 7 days after TBI (20 rats per group). Rats were sacrificed 14 days after TBI, and brain tissues were harvested for immunohistochemical studies, Western blot analyses, laser capture microdissections, and quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) to evaluate neurocan protein and gene expressions after various treatments.

Results. Animals treated with scaffold + hMSCs after TBI showed increased axonal and synaptic densities compared with the other groups. Scaffold + hMSC treatment was associated with reduced TBI-induced neurocan protein expression and upregulated growth-associated protein 43 (GAP-43) and synaptophysin expression in the lesion boundary zone. In addition, animals in the scaffold + hMSC group had decreased neurocan transcription in reactive astrocytes after TBI. Reduction of neurocan expression was significantly greater in the scaffold + hMSC group than in the group treated with hMSCs alone.

Conclusions. The results of this study show that transplanting hMSCs with scaffolds enhances the effect of hMSCs on axonal plasticity in TBI rats. This enhanced axonal plasticity may partially be attributed to the downregulation of neurocan expression by hMSC treatment after injury.

KeY Words • bone marrow stromal cells • neurocan • scaffolds • traumatic brain injury

Bone marrow stromal cells (MSCs) are a heterogeneous subpopulation of bone marrow cells that includes mesenchymal stem and progenitor cells. Extensive research conducted during the last decade has shown great promise for MSCs as an effective therapy for traumatic brain injury (TBI) in experimental models.32-35,37-41,52 and potentially in clinical settings. Also the underlying mechanisms of action of MSCs have been demonstrated to be neuro-restorative rather than neuro-substitutive.33 Among their neural remodeling effects, MSCs have been shown to promote axonal sprouting in the brain and spinal cord.42,60 The present study focuses on the ability of MSCs to inhibit neurocan, one of the growth-inhibitory molecules (growth-IMs) that suppress axonal regeneration after neural injury.18,25

The molecular mechanisms involved in axonal regeneration after neural injury are still unclear; however, over the last few years growth-IMs that have a repulsive effect

Abbreviations used in this paper: ANOVA = analysis of variance; CCD = charge-coupled device; CSPG = chondroitin sulfate proteoglycan; FITC = fluorescein isothiocyanate; GAP-43 = growth-associated protein 43; GFAP = glial fibrillary acidic protein; GMP = Good Manufacturing Processes; growth-IM = growth-inhibitory molecule; hMSCs = human marrow stromal cells; LCM = laser capture microdissection; MCID = micro computer imaging device; MSC = bone marrow stromal cell; NF-H = neurofilament H; qRT-PCR = quantitative real-time reverse transcriptase-polymerase chain reaction; TBI = traumatic brain injury.
on axonal regrowth have been identified. Neurocan is one of the well-studied growth-IMs and is present primarily in the axonal scar after neural injury. This study investigates the effect of administration of MSCs on neurocan expression after TBI.

In the present experiments, human MSCs (hMSCs) impregnated into collagen scaffolds were administered in the lesion core. We have employed different routes (intraarterial, intravenous, and intracranial) to administer MSCs into injured brain, and although they have all shown efficacy in improving functional outcome after TBI there have been problems with modes of administration. For example, relatively few MSCs can be injected intracranially, and the lesion core rapidly changes into a cavity that lacks the structural substrate to adequately support an inoculum of cells. Intravenous injection of MSCs via an internal carotid artery can cause embolic infarcts, whereas intravenous administration results in body-wide distribution of MSCs. Therefore, in an effort to optimize MSC therapy of TBI, we have recently used MSC-impregnated collagen scaffolds as MSC delivery vehicles.

Our studies have shown that transplanting MSCs after imregnating them into collagen scaffolds increases their concentration and survival at the injury site and provides superior functional outcome compared with treatment with MSCs alone. The fate of transplanted MSCs has been studied until 1 month after transplantation, and their number is significantly greater when they are transplanted with scaffolds than when they are administered alone. To revalidate the superior efficacy of scaffold + MSC administration, we have employed 2 treatment groups in this study: one receiving MSCs in combination with scaffolds and the other receiving MSCs alone. Collagen scaffolds have shown no beneficial effect when administered alone after TBI. They behave as inert delivery vehicles; therefore, we did not include a group treated with scaffolds alone.

Methods
Animal Model and Experimental Groups

This study was approved by the Henry Ford Health System Care of Experimental Animals Committee. Sixty adult male Wistar rats weighing 275–300 g were anesthetized with 4% chloral hydrate (350 mg/kg, administered intraperitoneally). A controlled cortical impact device was used to induce injury. Rats were placed in a stereotactic frame, and two 10-mm-diameter craniotomies were performed adjacent to the central suture, midway between lambda and bregma. The second craniotomy allowed for the lateral movement of brain tissue. The dura mater was not opened, and injury was induced by striking the left cortex covered with intact dura with a 6-mm-diameter tip pneumatic piston at a rate of 4 m/sec and 2.5 mm of compression. Velocity was measured using a linear velocity displacement transducer. The impact breached the dura, exposing injured brain, and different treatments were subsequently administered directly into the injured brain.

The study animals were randomly divided into 3 groups (20 rats per group). All animals were initially subjected to TBI and received one of the following treatments: 1) saline; 2) hMSCs alone; or 3) scaffold + hMSCs. Rats in the first group received a saline injection into the lesion cavity. Rats in the second group were injected with 3 × 10^6 hMSCs into the lesion cavity. In the third group, scaffolds impregnated with hMSCs (3 × 10^6 hMSCs per scaffold) were transplanted into the lesion cavity. After the treatments, the exposed cortex was covered with Gelfoam. The hMSCs were provided by Theradigm (a GMP-certified facility) and isolated and expanded, as previously described. Ultrafoam scaffolds (collagen Type I) were obtained from commercial sources (Davol). Seeding of hMSCs on scaffolds was performed as described previously. All treatments were performed 7 days after TBI. All rats were killed 14 days after TBI. The investigators performing different outcome measurements were completely blinded to study group assignment.

Tissue Preparation

For histological studies, rats were anesthetized with 4% chloral hydrate administered intraperitoneally and perfused transcardially with saline followed by 4% paraformaldehyde. Brains were harvested, postfixed in 4% paraformaldehyde at 4°C for 2 days, and then cut into seven 2-mm-thick standard coronal blocks on a rodent brain matrix. A series of sections were cut with a microscope through each of the 7 standard sections. For Western blot analysis, the ipsilateral cortical tissue from the lesion boundary zone was dissected, frozen in liquid nitrogen, and stored at −80°C. For laser capture microdissection (LCM), brain coronal sections were cut on a cryostat set at −20°C and kept at −80°C until processing.

Immunohistochemistry

After rehydration, tissue sections were treated with 3% H2O2 to block endogenous peroxidase activity and then boiled in 1% citric acid buffer (pH 6) in a microwave oven for 10 minutes. Sections were incubated in 1% bovine serum albumin for 1 hour to block the non-specific signals. Using the same buffer solution, the sections were incubated at 4°C with primary antibody (anti-synaptophysin 1:1000, Millipore; anti–growth-associated protein 43 [GAP-43] 1:200, Abcam; and a monoclonal antibody against pan-axonal neurofilament H [SMI 312] 1:1000, Covance) overnight. For negative controls, primary antibodies were omitted. For neurofilament H (NF-H) staining, sections were incubated with corresponding fluorochrome-conjugated secondary antibody (fluorescein isothiocyanate [FITC] 1:200, Jackson ImmunoResearch) at room temperature for 2 hours. For GAP-43 and synaptophysin staining, sections were incubated with corresponding fluorochrome-conjugated secondary antibody (1:200, Dako), followed by incubation with an avidin-biotin-peroxidase system (ABC kit, Vector Laboratories). DAB was then used as a sensitive chromogen for light microscopy. Tissue sections were mounted with cover slides and Vectashield mounting medium (Vector Laboratories). Negative controls consisted of sections treated identically except that the neurocan primary antibody was omitted. All immunohistochemical analyses were performed at the same time point by the same investigator; the negative control specimens were
Marrow stromal cells and neurocan suppression

prepared under identical conditions in the same batch runs.

For GFAP/neurocan double immunostaining, sections were incubated with a primary antibody cocktail of rabbit anti-GFAP (1:2000, Dako) and mouse anti-neurocan (1:200 Millipore) at 4°C for 3 days. Sections were subsequently incubated with a mix of FITC-conjugated (rabbit) and CY3-conjugated (mouse) secondary antibodies at room temperature for 2 hours. After washing with phosphate-buffered saline, sections were counterstained with DAPI and mounted with cover slides and Vectashield mounting medium (Vector Laboratories).

For quantitative measurement of the density of the immunoreactive area, microphotographs from the lesion boundary zone were digitized under a 20× objective using a 3-CCD color camera (Nikon) interfaced with a Micro Computer Imaging Device (MCID) image analysis system (Imaging Research, Inc.).11,14 We drew the lesion boundary zone areas on coronal sections using a low-power objective. A higher power was then selected, and the MCID system used random systematic sampling to sample 30% of the defined region. When the system moved to the first location within the region of interest, a counting frame was placed over the selected area. We then counted the positive area of immunostained cells within the counting frame. The data collected from 5 sections and 8 fields within each section were averaged to obtain a single value for 1 animal and were presented as the percentage of positive area for synaptophysin, NF-H, and GAP-43 immunoreactive profiles. Data were analyzed by a researcher who was blinded to the animals’ group assignments.

Western Blot Analysis

Rats were killed at Day 14 after TBI. Brain tissue from the cortical lesion boundary zone was collected, washed once in phosphate-buffered saline, and lysed in lysis buffer (20 mM Tris pH 7.6, 100 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 1% deoxycholic acid, 10% glycerol, 1 mM EDTA, 1 mM NaVO3, 50 mM NaF, Protease Inhibitor Cocktail Set I from Calbiochem). After sonication, soluble protein was obtained by centrifugation at 13,000 × g at 4°C for 15 minutes. Total protein concentrations were determined with bicinchoninic acid (BCA) protein assay (Pierce). Equal amounts of lysate were subjected to SDS-polyacrylamide electrophoresis on Novex Tris-glycine precast gels (Invitrogen), and separated proteins were then electroblotted to polyvinylidene fluoride (PVDF) membranes (Millipore). After exposure to various antibodies, specific proteins were visualized using the SuperSignal West Pico Chemiluminescent Substrate system (Thermo Scientific). The band intensity was analyzed using Scion Image software (Scion). Antibodies used for Western blot included anti-synaptophysin (1:4000, Millipore), anti–pan-axonal nervefilament H (SMI 312) (1:1000 Covance), anti–GAP-43 (1:10,000, Abcam), anti-neurocan (1:2000, Millipore), and anti-actin (1:5000, Santa Cruz Biotechnology).

Laser Capture Microdissection

Laser capture microdissection (LCM) was used to harvest astrocytes in the injured hemisphere from cryostat coronal brain sections. GFAP immunostaining was used to identify reactive astrocytes.54 The sections localized to the lesion boundary zone were air-dried for 30 seconds and fixed in freshly prepared cold acetone at 4°C for 2 minutes. The sections were incubated with GFAP antibody at 1:100 dilution for 7 minutes, followed by incubation with CY3-conjugated secondary antibody for 7 minutes. After air-drying for 5 minutes, GFAP-positive (reactive) astrocytes along the lesion boundary were dissected using the Leica LMD6000 system (Leica Microsystems). Reactive astrocytes were identified by their intense GFAP staining, cellular hypertrophy, and increased process extension. Approximately 1000 cells were collected in RNase-free tubes containing 50 μl of lysis buffer, and the samples were stored at −80°C before RNA isolation.

Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using the SYBR Green system. Total RNA was isolated from laser-captured cells using the RNeasy Micro Kit (Qiagen). RNA integrity number values and concentrations were assessed immediately after purification by electrophoresis. Total RNA was then stored at −80°C until use. Complementary DNA was subsequently synthesized from RNA using a SuperScript III Platinum reverse transcriptase kit (Life Technologies). Quantitative real-time RT-PCR was performed afterward. Gene-specific primers were designed with Primer 3 (Life Technology), and concentrations (10 nM) were optimized before use. A SYBR Green PCR master kit was used with the appropriate concentrations (10 nM) of forward and reverse primers in a total volume of 20 μl. PCR reactions contained 2 μl cDNA. Quantitative real-time RT-PCR was performed on a Viit7 PCR instrument (Life Technologies). Specificity of the produced amplification product was confirmed by examination of dissociation reaction plots. A distinct single peak indicated that a single DNA sequence was amplified during qRT-PCR. Quantitative real-time RT-PCR products were run on 2% agarose gels to confirm that the correct molecular sizes were present. Each sample was tested in triplicate with qRT-PCR, and samples obtained from multiple animals were used for analysis of relative gene expression data using the 2−ΔΔCt method. Primers were designed to contain a GC content of 45%–55% and to span introns to hinder amplification of genomic DNA. Primers employed were as follows: β-actin: 5′-CCA TCA TGA AGT GTG ACG TTG-3′ (forward), 5′-CAA TGA TCT GTA TCT TCA TGG TG-3′ (reverse). Neurocan: 5′-AGA ATG GAC GCT GGA ATG A-3′ (forward), 5′-CAT CAC ACT GGT ATC GCA CA-3′ (reverse).

Statistical Analyses

All data are presented as means with SDs. Data were analyzed by a 1-way analysis of variance (ANOVA) test followed by a post hoc Student-Newman-Keuls (SNK) test to assess the between-group differences. Statistical significance was present when p values were less than 0.05.
Results

Increase in Axonal and Synaptic Density After TBI

Axons were identified with fluorescent immunostaining of a pan-axonal NF-H marker, SMI 312. TBI-reduced axonal density was shown as low NF-H–positive signals in the lesion boundary zone (Fig. 1A). Treatment with hMSCs alone resulted in increased NF-H density compared with the density in animals from the saline control group (Fig. 1B and D, p < 0.05 vs saline). In addition, animals in the scaffold + hMSC group showed significantly increased density of NF-H-positive axons compared with the saline and the hMSC-alone groups (Fig. 1C and D, p < 0.05 vs saline, p < 0.05 vs hMSC alone). Axonal sprouting was also studied with GAP-43 immunostaining. TBI induced low-level GAP-43 expression in the injured area. Animals treated with scaffold + hMSC treatment showed a significant increase in GAP-43 expression compared with the saline control group, whereas those treated with hMSCs alone showed no increase (Fig. 1E–H, p < 0.05 vs saline, p < 0.05 vs hMSC alone).

Synaptophysin was employed to measure the level of synapses after TBI. TBI led to loss of synapses in the lesion boundary zone, which was not reversed by treatment with hMSCs alone (Fig. 1I, J, and L). Specimens from animals in the scaffold + hMSC group, however, showed increased synaptophysin-positive signals (Fig. 1K). Quantitative analysis reveal a significant increase in synaptophysin density in the peri-injury area in animals in the scaffold + hMSC group compared with the saline and hMSC-alone groups (Fig. 1L, p < 0.05 vs saline, p < 0.05 vs hMSC alone).

Western blot analysis using tissue dissected from the cortical lesion boundary area confirmed these protein expression patterns (Fig. 2 left). Scaffold + hMSC treatment was associated with a significant increase in the NF-H, GAP-43, and synaptophysin protein levels compared with saline control (Fig. 2 right, p < 0.05 vs saline). In addition, scaffold + hMSC treatment was superior to hMSC-alone treatment in inducing expression of NF-H and synaptophysin after TBI (Fig. 2 right, p < 0.05 vs hMSC alone).

Reduction of Neurocan Protein Level in Reactive Astrocytes of the Lesion Boundary Zone

Neurocan is mainly expressed in reactive astrocytes, therefore double immunostaining with GFAP/neurocan was performed to investigate neurocan expression with various treatments. Reactive astrocytes were identified by GFAP with dense staining of the enlarged cell bodies and the highlighted cell processes. TBI induced prominent neurocan expression in the lesion boundary zone. Neurocan staining signals were mainly dispersed in the extra-cellular matrix, while some were colocalized with GFAP-positive astrocytes (Fig. 3A and D) in the lesion boundary area. Animals treated with hMSCs alone showed decreased neurocan expression in reactive astrocytes (Fig. 3B, E, and G, p < 0.05 vs saline). Furthermore, those treated with scaffold + hMSCs showed greater reduction of the neurocan level in the reactive astrocytes than those treated with hMSCs alone (Fig. 3C, F, and G, p < 0.05). Western blot analysis of cortical tissues dissected from the lesion boundary zone confirmed that while TBI induced neurocan expression, scaffold + hMSC treatment was associated with a significant reduction in the level of neurocan (Fig. 4, p < 0.05 vs saline, p < 0.05 vs hMSC alone).

Reduction of Neurocan Gene Expression in Reactive Astrocytes

In order to further investigate whether scaffold + hMSC–induced neurocan protein reduction results from the downregulation of genes, GFAP staining and LCM were performed to identify and dissect reactive astrocytes. Approximately 1000 reactive astrocytes were collected from the lesion boundary zone, and neurocan gene expression was detected by qRT-PCR (Fig. 5A–C). Quantitative real-time RT-PCR results showed decreased neurocan gene expression in animals treated with hMSC alone (Fig. 5D, p < 0.05 vs saline) and further reduction in those in the scaffold + hMSC treatment group (Fig. 5D, p < 0.05 vs saline, p < 0.05 vs hMSC alone). These data are consistent with the pattern of neurocan protein expression alteration detected by immunostaining and Western blot analysis, suggesting that scaffold + hMSC treatment affects neurocan expression from the gene level.

Discussion

Our results show that hMSC administration after TBI decreases neurocan expression and increases axonal density in injured brain. Also, administration of hMSCs in combination with scaffolds was more effective in suppressing neurocan levels than administration of hMSCs alone. We have previously shown functional benefits of hMSC treatment after TBI.34,42,52,60 The present study was designed to gain insight into the molecular and structural basis of this benefit; therefore, the effects on suppression of growth-IM (neurocan) and induction of axonal plasticity was studied. Neurocan expression was studied at gene (qRT-PCR) and protein levels (Western blot analysis), whereas axonal plasticity was studied using NF-H and synaptophysin staining. We also employed staining for GAP-43, which is a membrane-bound protein found in growth cones of developing and sprouting axons and has been used as an index of axonal regeneration.1,23,44 However, because it is nonspecific and also expressed by astrocytes and other cell types, its validity as a marker of axonal regeneration has been challenged.56 Notwithstanding, our results showed increased axonal and synaptic densities, indicating induction of axonal plasticity even if positive GAP-43 data cannot be considered as unequivocal evidence of axonal regeneration.

In the adult mammal, the injured CNS is a highly inhibitory environment for axonal regrowth.38 This limitation is due to a lack of neurotrophins, but also due to the presence of growth-IMs.60 These growth-IMs include chondroitin sulfate proteoglycans (for example, neurocan, versican, phosphacan, and brevican),22 Nogo-A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp).25 Their role as major impediments of neurite outgrowth was first recognized in the 1980s4,9,13 These growth-IMs are present in normal brain and spinal cord, but they are upregulated after spinal cord inju-
Marrow stromal cells and neurocan suppression

The neurons in the CNS have a limited capacity to regenerate after an injury in contrast to the peripheral nervous system. However, it was recognized more than two decades ago that CNS axons can regenerate when provided with a permissive environment. This led to several experimental studies utilizing inhibitors of growth-IMs in spinal cord injury and ischemia models of CNS injury. Late ly, the same strategy has been used in experimental TBI studies using antibodies against growth-IMs. MSCs have been shown to block the effects of these growth-IMs and promote neurite outgrowth in vitro models of CNS injury. The suppressive effect of MSCs on the growth-IM neurocan has also been demonstrated in an in vivo model of cerebral ischemia, and it has been hypothesized that the MSCs promote axonal regeneration by inhibiting the effect of neurocan. Furthermore, neurotrophic factors, such as BDNF, which are induced by MSCs, also act as suppressants of growth-IMs. There has been no study to examine the effects of MSC administration on

Fig. 1. Effects of scaffold + hMSC (S+hMSC) treatment on axonal density in the injured cortex. Axonal density is illustrated by immunostaining with NF-H (A–C) and GAP-43 staining (E–G). Synaptic density is shown by synaptophysin (SYP) immunostaining (I–K). Bar graphs (D, H, and L) show the quantitative data of the immunostainings (n = 8). Data represent mean ± SD. Bars = 50 μm. *p < 0.05 vs saline group; #p < 0.05 vs hMSC-alone group.

Fig. 2. Representative Western blot (left) and densitometry data (right) of NF-H, GAP-43, and synaptophysin in the cortical lesion boundary zone (n = 4). Data represent mean ± SD. *p < 0.05 vs saline group; #p < 0.05 vs hMSC-alone group.
neurocan expression in TBI models, and our research is the first investigation on this subject.

Neurocan is a multidomain hyaluronan-binding chondroitin sulfate proteoglycan (CSPG) that plays an important regulatory role in axonal regeneration. In the embryonic life neurocan is synthesized by neurons and controls axonal guidance during brain development. It acts via a repulsive mechanism, and it has been suggested that presence of neurocan in the embryonic spinal cord prevents incoming dorsal root ganglion axons from crossing the midline and that neurocan is involved in the formation of barrel fields in the somatosensory cortex. The concentration of neurocan increases during embryonic development but then rapidly declines during the early postnatal period. Full-length neurocan is only expressed in the juvenile brain, whereas in the adult brain only neurocan fragments are detectable. However, evidence from various experimental models of CNS injury (for example, kainate-induced seizures, ischemia, and TBI) indicates that neurocan is upregulated and full-length neurocan reaccumulates in the adult life around the injury site. Also, in contrast to embryonic life, during which neurocan is synthesized by neurons, in adult animals neurocan is primarily synthesized by reactive astrocytes and is expressed in the glial scar. The reactive astrocytes play a major role in the formation of glial scar after neural injury, and this glial scar acts not simply as a mechanical barrier to regenerating axons but also inhibits axonal regeneration at the biochemical level by the presence of growth-IMs such as neurocan. The significance of inhibiting the role of CSPGs on axonal regrowth is confirmed by the fact that treatment with the proteolytic enzymes that leave CSPGs such as chondroitinase ABC promotes axonal regrowth both in vitro and vivo. The injection of chondroitinase ABC into the injury site increased axonal density and functional recovery in rats after spinal cord injury and nigrostriatal lesions. In our present study, MSCs manifested an effect similar to chondroitinase ABC (that is, by inhibiting neurocan they promoted axonal regeneration). It has been shown in cerebral ischemia studies that MSCs modulate poststroke astrogenic response and decrease the thickness of scar wall, which enhances brain repair after ischemia. However, we appreciate that MSCs have several other neurorestorative properties, such as suppression of other growth-IMs and induction of neurotrophic factors, which also contribute to neural recovery after TBI, and the beneficial effects of MSC administration are not solely due to their ability to inhibit neurocan.
Conclusions

By demonstrating that administration of MSCs, particularly MSCs impregnated into collagen scaffolds, reduces neurocan expression at both the gene and the protein level, the current study further validates the importance of growth-IMs in preventing neurorestoration after neural injury and also emphasizes the beneficial role of MSCs in suppressing growth-IMs and promoting neural repair. This enhances our understanding of the mechanisms of action of MSCs and also adds to our knowledge of the biology of neural injury and repair.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following: Conception and design: A Mahmood, Xiong, Kaplan, Chopp. Acquisition of data: Wu, Qu, S Mahmood, Xiong. Analysis and interpretation of data: A Mahmood, Wu, Qu, S Mahmood, Xiong, Kaplan. Drafting the article: Wu, Xiong. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: A Mahmood. Administrative/technical/material support: Chopp. Study supervision: A Mahmood, Chopp.

References


McKeon RJ, Höke A, Silver J: Injury-induced proteoglycans

A. Mahmood et al.
Marrow stromal cells and neurocan suppression


---

Manuscript submitted June 26, 2013. Accepted December 3, 2013. Please include this information when citing this paper: published online January 24, 2014; DOI: 10.3171/2013.12.JNS131362. Address correspondence to: Asim Mahmood, M.D., Henry Ford Hospital, Neurosurgery Department, 2799 West Grand Blvd., Detroit, MI 48202, email: AMAHMOO2@hfhs.org.