Spinal Macrophage Migration Inhibitory Factor Is a Major Contributor to Rodent Neuropathic Pain-like Hypersensitivity

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ABSTRACT

Background: Neuropathic pain-like hypersensitivity evoked by peripheral nerve injury is a salient clinical feature of pathologic pain; however, the underlying mechanisms of this condition remain largely unknown. Previous work has confirmed that spinal macrophage migration inhibitory factor (MIF) contributes to the pathogenesis of formalin-induced inflammatory hyperalgesia, but the role for MIF in neuropathic pain is still not well defined.

Methods: After approval by the Ethical Committee of Animal Use and Care, the sciatic chronic constriction nerve injury-induced rodent model of neuropathic pain was built. The mechanical threshold with von Frey hairs and thermal latency with hot plate were measured, and the expression of spinal MIF, CD74, and downstream extracellular signal-regulated kinase 1/2 signaling cascade was detected. Finally, MIF gene mutation and exogenous recombinant MIF were used for further clarification.

Results: Intrathecal MIF tautomerase inhibitor reversed sciatic chronic constriction nerve injury-induced pain behaviors. The expression of MIF and CD74 was up-regulated in a time-dependent manner in the ipsilateral spinal cord dorsal horn. These changes were associated with the activation of extracellular signal-regulated kinase 1/2 signaling cascade. In mice, sciatic nerve injury that resulted in hypersensitivity was associated with an upregulation of MIF in the spinal cord. Inhibition of MIF pharmacologically or genetically reduced hypersensitivity, suggesting a role in neuropathic pain.

Conclusions: MIF-associated extracellular signal-regulated kinase 1/2–N-methyl-D-aspartic acid receptor ex-pression and additional production of prostaglandin E₂. Further, MIF gene mutation and exogenous recombinant MIF could desensitize and mimic sciatic chronic constriction nerve injury-evoked pain responses and cellular changes, respectively.

PAIN protects individuals from actual or potential body injury, but pain resulting from a primary lesion or dysfunction of the nervous system (i.e., neuropathic pain) plays no obvious biologic role in producing a persistent hypersensitiv-ity to externally normally noxious (hyperalgesia) and innocuous...
stimuli (alldynia).1–3 Despite increasing knowledge on neuropathic pain in basic science, it is often difficult to treat clinically with currently available therapeutics.4 The mechanisms underlying neuropathic pain developing from nerve injury remain largely unknown. In the past decade, research has provided a body of evidence upon the association of nerve injury with mobilized immune reactions.5–7 Infiltration of T cells,8 recruitment of leukocytes,9 activation of microglia (the resident macrophage in the central nervous system),10 and release of pro- and antiinflammatory cytokines11 are initiated in the central nervous system in the early period of nociceptive stimuli. In addition, activated glia and the immune system contribute to the development and maintenance of neuropathic pain.12 A cross talk between inflammatory responses and proinflammatory mediators, such as inflammatory cytokines, has been considered as an important component of both conditions of acute and chronic pain.13

Macrophage migration inhibitory factor (MIF), a proinflammatory cytokine first described in the 1960s, is a structurally unique homotrimer among cytokine proteins, with each monomer consisting of two α-helices and a four-stranded β-sheet. MIF is similar to the bacterial isomerize enzyme, D-dopachrome-tautomerase, based on its ability to convert D-dopachrome to 5,6-dihydroxyindole-2-carboxylic acid.14 Recent studies suggest targeting MIF tautomerase activity could provide a new, focused therapeutic strategy for inflammation-associated diseases, as inhibitors of tautomerase activity reduce MIF biologic function.15 MIF is currently known to play critical roles in both innate and adaptive immune responses by binding to the extracellular domain of CD74 and/or the noncognate CXC chemokine receptor, CXCR4.16,17 The proinflammatory property of MIF is increasingly recognized as a fundamental constituent of many pathologic conditions, including traumatic inflammation, sepsis, tumor, obesity, autoimmunopathies, cardiopathies, and neural degenerative diseases.18 With the exception of MIF as a proinflammatory cytokine and a highly active enzymatic isomerase, it also functions like an anterior pituitary hormone and a major histocompatibility complex class I factor.19–21 Several lines of evidence indicate that MIF is a crucial mediator of neural functions, and suggest a link between MIF and nerve injury,22 which may be responsible for pain-associated behavioral responses.

A significant level of the baseline and inducible MIF expression by proinflammatory stimuli in the neurons of the hippocampus as well as in other regions of the brain implicates the neuronal function of MIF in the central nervous system.23 The increased levels of MIF in both proximal and distal segments of thetransected sciatic nerve and the positive staining of MIF with Wallerian degeneration in neural fibers indicate MIF not only presents in the peripheral nerves,24 but also plays an essential role in accelerating peripheral nerve regeneration and preventing Schwann cell apoptosis by inhibiting p53.25 Nevertheless, the abnormal expression of the MIF gene after different types of axonal injuries is in association with an induced motoneuron death after spinal cord ventral root avulsion in adult animals,26 and MIF can induce dysfunction of spinal cord neurons and lead to cell death through oxidative stress.27 A more recent study showed that neuronal survival can be hindered by the existence of MIF after spinal cord injury, and the detrimental secondary molecular responses in this pathologic context can be alleviated if MIF is blocked.28 Due to the link between peripheral nerve injury and pain,29,30 we confirmed a pathologic relationship between MIF and inflammatory pain behaviors in rats,31 but whether or not there is a role for MIF in mechanisms of neuropathic pain due to peripheral nerve injury has yet to be clarified.

Considering MIF exerts its biologic functions through binding to the putative membrane receptor, CD74, and induces downstream activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling cascade,32 we hereby hypothesized that spinal MIF might take an essential part in the underlying mechanisms of peripheral nerve injury-induced neuropathic pain through MIF-associated downstream cellular signaling.

Materials and Methods

Experimental Subjects

This study was approved by the Institutional Committee of Animal Care and Use (Nanjing University, Nanjing, China), and all experiments were performed under the guidance of the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals. Male C57BL/6 mice weighing 20–25 g, 7–9 weeks of age, and wild-type mice (The Jackson Laboratory, Bar Harbor, ME) were used for all behavioral tests. The perioperative treatment of the animals was reported previously.31 In brief, a reverse 12:12-h dark-light cycle was used on animals, and the housing temperature was maintained at 23 ± 1°C. After randomization, each animal was placed into a box with three mirrored sides for 10 min, and then the test sessions took place. After the experiment, a lethal dose of pentobarbital was administered to euthanize the animal.

Testing Drugs

Given MIF possesses tautomered properties,20 one of the earliest reported MIF inhibitors, (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid, methyl ester (ISO-1; Merck KGaA, Darmstadt, Germany), was used in this study to inhibit the catalytic site of MIF, leading to a marked reduction in the biologic function of MIF.33 ISO-1 was dissolved in 10% dimethyl sulfoxide (DMSO) (which diluted in normal saline). The diluted DMSO was used as the vehicle control. In addition, to test the role of MIF in naïve mice for regulating the pain behavioral responses, mouse recombinant MIF (rMIF) was purchased from R&D Systems (Minneapolis, MN), and the dilution saline was used as the vehicle comparison.
**Intrathecal Catheterization**

Mice were implanted with an intrathecal catheter (PE-10 tubing; Scientific Commodities Inc., Lake Havasu City, AZ) for drug delivery as described previously. In brief, after shaving and sterilizing the surgical area, a midline incision was made, and paravertebral muscles were reflected from the spinous processes. Under the guidance of a surgical microscope, a hole (1 × 1 mm) was drilled manually until the dura was exposed. The dura was slit and a 25-gauge needle was traversed, which resulted in leakage of clear cerebrospinal fluid (CSF). After the drug-administration catheter was placed, the catheter was fixed with a drop of tissue glue (Histocryl; B. Braun, Tuttlingen, Germany) and further, was secured on the fascia of paravertebral muscle. Finally, sodium penicillin (10,000 IU; Shanghai AoBopharmtech, Shanghai, China) was given intramuscularly. The mice would be excluded if neurologic deficits were exhibited for reaction to the thermal stimuli. The maximal duration of stimulation was set at 22 s as a cutoff time to avoid tissue damage. Each animal was tested three times repeatedly at an interval of 15 min, and each time, the test was carried out by a different investigator. Finally, the means and the SDs of the three tests were calculated.

Baseline values of mechanical and thermal stimuli were recorded 2 days before ISO-1 or rMIF treatment, which was used prophylactically 30 min before CCI or sham surgery.

**Electrophysiology**

The spinal cord was quickly removed and placed in ice-cold artificial CSF (pH = 7.40), which contained sucrose (234 mM), CaCl₂ (2.5 mM), KCl (3.6 mM), MgCl₂ (1.2 mM), NaHCO₃ (25 mM), NaH₂PO₄ (1.2 mM), and glucose (12 mM) and was bubbled continuously by a mixture of 95% O₂/5% CO₂. The spinal cord transverse slices (500 μm) were prepared with a vibratome (Camden Instruments, London, United Kingdom). Then, the slices were buried in standard artificial CSF (NaCl 117 mM, KCl 4.7 mM, NaH₂PO₄ 1.2 mM, CaCl₂ 2.5 mM, MgCl₂ 1.2 mM, NaHCO₃ 25 mM, and glucose 11 mM; pH = 7.40) for more than 1 h at room temperature. Subsequently, a single slice was transferred to a recording chamber with perfused artificial CSF at a speed of approximately 2–3 ml/s. The same procedure was carried out for the vehicle control.

Monosynaptic excitatory postsynaptic currents (EPSCs) of the spinal cord slices were recorded using the whole cell current- and voltage-clamp recordings. The evoked EPSCs on the substantia gelatinosa neurons, a major projection site of small-diameter afferent nerve fibers that predominantly transmit nociceptive signals, were assessed as per previously reported methods. In brief, the electrodes were made from borosilicate glass (1.5 mm in OD; Hilleggen, Malsfeld, Germany) with a tip impedance of approximately 3–6 MΩ. The internal pipette solution contained (in mM): 122°K gluconate, 5 NaCl, 0.3 CaCl₂, 10 HEPES, 2 MgATP, 0.1 Na₂GTP, 5 BAPTA, and 1 EGTA; pH was 7.30. The osmolality of the pipette fluid was 300 mOsm. Under visual control (40× objective and 10× ocular magnification), the electrode was positioned toward the center of the substantia gelatinosa. Once a patch electrode was sealed on the cell tightly (more than 1 GΩ), the neurons were included in the sample if the testing membrane potential was more negative than −50 mV and action potentials overshooting 0 mV evoked by direct depolarizing current injection. No compensation was made to the shift potential caused by liquid junction. The pCLAMP 9 software (Molecular Devices, Inc., Sunnyvale, CA) was used for data acquisition and analyses. The accepting recordings resistance should be less than 2.5 times the pipette resistance.

In the spinal dorsal root stump near the dorsal root entry zone, a concentric bipolar stimulus electrode (60 μm in diameter) was placed and stimulated with a focal stimulation. Electrical stimuli (150 μs square-wave pulses) were applied for reaction to the thermal stimuli. The maximal duration of stimulation was set at 22 s as a cutoff time to avoid tissue damage. Each animal was tested three times repeatedly at an interval of 15 min, and each time, the test was carried out by a different investigator. Finally, the means and the SDs of the three tests were calculated.

Baseline values of mechanical and thermal stimuli were recorded 2 days before ISO-1 or rMIF treatment, which was used prophylactically 30 min before CCI or sham surgery.
at frequencies of less than 0.25 Hz. An input-output curve was determined at a holding potential (~60 mV) by increasing stimulus intensities from 50 to 150 μA given once every 10 s. The evoked EPSCs were recorded and judged to be monosynaptic on the basis of stable latencies of the EPSC peak amplitude. The Mini Analysis Program 6.0.3 (Synaptosoft, Inc., Decatur, GA) was used to determine the frequency and amplitude of EPSCs.

**Enzyme-linked Immunosorbent Assay (ELISA) for MIF and Prostaglandin E2 (PGE2)**

The sample of CSF (0.2 ml) was collected from the implanted intrathecal catheter, and blood (0.5 ml; centrifuged for plasma) was collected from a catheterized femoral vein. After sample collection each time, some volumes of physiologic saline were infused to CSF or blood as corresponding replacements. MIF levels in CSF and plasma were detected using ELISA according to the manufacturer’s protocol. In brief, 96-well plates (Nunc GmbH, Wiesbaden, Germany) were coated with rabbit antismouse MIF (Abcam, Cambridge, MA). Nonspecific binding sites were blocked by the addition of 250 μl phosphate-buffered saline containing 1% bovine serum albumin/5% sucrose/0.05% NaN3 and incubation for 16 h at 4°C. After plates had been washed three times, recombinant mouse MIF standard and test sera were added to the wells and incubated for 2 h. Biotinylated polyclonal rabbit antimouse MIF was used as the detection antibody, and streptavidin-horseradish peroxidase (HRP; Jackson/Dianova GmbH, Hamburg, Germany) was used as the second-step reagent. Color was developed with 3,3′,5′,tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO), and absorbance was measured at 450–620 nm against standard curves. All analyses were performed in duplicate, and the means ± SD were reported. The detection limit of the MIF assay was 0.015 ng/ml.

PGE2 was detected using a newly developed specific Enzyme Immunoassay Assay kit (DetectX®, ArborAssays, Ann Arbor, MI). In brief, a standard curve was generated according to the kit’s guidance. The CSF sample was diluted in a 1:10 ratio with the provided assay buffer, which was pipetted into a clear microtiter plate coated with an antibody to capture mouse immunoglobulin G. Then, 25 μl PGE2-per-oxidase conjugate was added into the wells and followed by adding 25 μl monoclonal antibody to PGE2 to each well and shaken for 15 min at room temperature. After an overnight incubation at 4°C, the plate was washed four times with washing buffer, and 150 μl tetramethylbenzidine substrate was added. After a 30-min incubation, the reaction was stopped by adding 50 μl stop solution, and the intensity of the generated color was detected in a microtiter plate reader capable of measuring a 450-nm wavelength. The concentration of the PGE2 in the sample was calculated using the suitable correction for the dilution of the sample. The detection sensitivity of PGE2 was less than 1.1 pg/well.

**Immunoblotting Analysis**

Mice were sacrificed by a lethal dosage of pentobarbital. The ipsilateral lumbar spinal cord dorsal horn was separated from the contralateral one using tissue microscopy and was homogenized in lysis buffer (10 mM Tris, 5 mM EDTA, 2% Triton X-100, 0.2 mM Na2VO4, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin and aprotonin) after being mechanically disrupted. Blots were blocked with 5% nonfat dry milk in phosphate-buffered saline with 0.1% Tween-20 and then incubated with one of the following primary antibodies: MIF (Acris Antibodies GmbH, Herford, Germany): 1:1,500, goat polyclonal against mouse; CD74 (SantaCruz Biotechnology, Inc., Santa Cruz, CA): 1:1,000, goat polyclonal against mouse; N-methyl-D-aspartic acid receptor type 2 subunit B (NR2B; R&D Systems): 1:1,000, rabbit polyclonal against mouse; ERK1/2 (p44/p42 mitogen-activated protein kinase [MAPK]; DB Biotech, Kosice, Slovak Republic): 1:5,000, rabbit monoclonal against mouse; phospho-p44/p42 MAPK (Cell Signaling Technology, Danvers, MA): 1:2,000, rabbit polyclonal against mouse; and interleukin (IL)-8 (Abcam): 1:5,000, rabbit polyclonal against mouse. After repeated washing, goat antirabbit secondary antibody (1:4,000, HRP-conjugated; Vector Laboratories, Peterborough, United Kingdom) or rabbit antigoat secondary antibody (Sigma-Aldrich) incubation was performed, developed with a chemiluminescence system, and followed with film exposure and relative density analysis with the Typhoon Imaging System (GE Healthcare, Piscataway, NJ). The immunoblots were washed briefly and then incubated with a polyclonal antimouse glyceraldehydes 3-phosphate dehydrogenase antibody (1:10,000; GenScript USA, Inc., Piscataway, NJ) or a monoclonal antimouse β-actin antibody (1:10,000; Sigma-Aldrich) for 40 min at room temperature, followed by an HRP-conjugated goat antirabbit antibody. The protein of glyceraldehyde 3-phosphate dehydrogenase or β-actin was then visualized as the loading biomarker.

**Immunohistochemistry**

Spinal cords were harvested at 14 days from the CCI or sham-treated mice and were fixed in 4% paraformaldehyde and 20% sucrose in PBS. Lumbar spinal cord segments were removed, postfixed for 2–6 h, and kept overnight in 15% sucrose. Transverse spinal cord sections (20 μm) were cut using a cryostat (n = 5–6 to each treatment). Sections were blocked with 4% goat serum in 0.3% Triton X-100 for 1 h at room temperature and incubated for 24 h at 4°C with MIF primary antibody (1:1,000, mouse polyclonal). Besides primary antibodies of CD74 (1:1,000), mouse monoclonal; CD11b (OX-42; 1:5,00 mouse monoclonal; ebiosciences, Inc., San Diego, CA), a molecule has been widely used as a marker for microglial identification31,41: glial fibrillar acidic
MIF Tautomerization and rMIF Activity Analyses

One major characteristic of MIF is the tautomerase activity, and blockade of this enzymatic activity can inhibit the biologic functions of MIF effectively. \(^{20,33}\) In this study, we used ISO-1 as the inhibitor of MIF tautomerase activity. Therefore, the tautomerase activity of MIF was evaluated further through a modified assay reported in our previous work and elsewhere. \(^{20,34}\) In brief, either mouse microglial cells (SciCell Research Laboratories, Carlsbad, CA) lysates, or mouse CSF after treatment with ISO-1 were used for this enzymatic activity analysis. In the first assay, mouse microglial cells (1 × 10\(^6\), repeated six times) were treated with ISO-1 ranging from 0.01 to 100 \(\mu\)M at 37°C for 30 min. The medium was then replaced with ISO-1-free medium, and the cells were washed and lysed with 600 \(\mu\)l ice-cold lysis buffer at 4°C for 20 min. In the second assay, the CSF from mice (n = 6) treated with saline, 10% DMSO, or ISO-1 (100 \(\mu\)g/kg per day in 10% DMSO) were collected at day 14 after CCI surgery. Both assays used L-dopachrome methyl ester prepared through oxidation of l-3,4-dihydroxyphenylalanine methyl ester with sodium periodate. Tautomerase activity was determined by adding dopachrome methyl ester 0.3 ml to a cuvette containing 0.7 ml the supernatants, and the spectrophotometric measurements were made at 475 nm for 20 s by monitoring the rate of decolorization of L-dopachrome methyl ester in comparison to a standard solution. Further, the levels of MIF in the mouse microglial cell lysates and the mouse CSF were measured using ELISAs.

Mouse-derived rMIF was used in normal mice to detect its role in nociceptive behaviors. Therefore, MIF activity was assessed using MIF-dependent phosphorylation of ERK1/2 (p44/p42 MAPK) measured using western blotting. The phospho-p44/p42 and total p44/p42 proteins were detected with specific antibodies. \(^{32}\) In addition, MIF-induced secretion of PGE\(_2\) was measured by specific ELISA. \(^{32,44}\) The tautomerase activity of MIF was measured by visible spectrophotometry using L-dopachrome methyl ester as a substrate. \(^{45}\)

Statistical Analyses

Data are presented as the means ± SDs and were analyzed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., San Diego, CA). Nociceptive data and multiple group results were analyzed with two-way ANOVA, where treatment and nociceptive status or other multiple factors were considered as independent variables in the model, and all of the factors were between-subjects factors. The ANOVA was always followed by the Bonferroni post hoc tests for multiple comparisons among different time points. When there were two testing groups, a Student \(t\) test was used to analyze the intergroup difference in the blood and tissue levels of MIF, PGE\(_2\), and other proteins. The expressions of proteins detected with immunohistochemistry were analyzed with a paired \(t\) test. The cumulative distribution of EPSC amplitude and frequency were analyzed with the Kolmogorov-Smirnov test. All reported \(P\) values are two-sided, and a \(P\) value of less than 0.05 was accepted for statistical significance.

Results

Spinal MIF Level Is Increased after CCI

Mechanically and thermally evoked pain behaviors in CCI mice displayed statistical difference between the ipsilateral and the contralateral paws, but this did not occur in the sham animals (fig. 1, A and B; \(P < 0.05\); n = 6 in the sham group and n = 8 in the CCI group). Based on this observation, we measured MIF content in mice plasma and CSF and found that MIF level was increased in the CSF on day 3 after CCI, but no change was observed in the plasma (fig. 1C; \(P < 0.01\); n = 6). Further, we detected MIF levels in the spinal cord dorsal horn using the immunofluorescence technique after CCI and sham surgeries. Although there was no significant difference in MIF content in both laterals of the spinal cord dorsal horn in the sham-treated mice, the level of MIF in the ipsilateral dorsal horn in the CCI mice was increased significantly, compared with the contralateral (fig. 1D; n = 6). Whether the increased MIF in spinal cord played roles or not need to be guaranteed. Therefore, we measured the electrophysiological property of the ipsilateral dorsal horn in the spinal cord sections, and found that MIF-specific inhibitor ISO-1 could block the firing activity of neurons in the spinal cord dorsal horn (fig. 1E; n = 8). These results indicate that the increased MIF in the spinal cord may be involved in the functional regulation of the pain behavioral responses.

Spinal MIF Critically Contributes to the CCI-evoked Pain Behaviors

Intrathecal administration of MIF inhibitor ISO-1 produced a dose-related inhibition of the CCI-induced mechanical and thermal pain responses (i.e., ISO-1 40 \(\mu\)g = 30 \(\mu\)g = 20 \(\mu\)g more than 10 \(\mu\)g = DMSO = saline) (fig. 2, A and B; n = 6 in the sham group and n = 8 in the CCI group), but ISO-1 itself in the sham animals played no role in the experimental pain behaviors (fig. 2, C and D; n = 6). To clarify the
inhibitory role of ISO-1 in the enzymatic effect of MIF that is functionally associated with its biologic role, we used anti-MIF monoclonal antibody intrathecally to verify the role of the increased spinal MIF and found a similar inhibitory effect produced by MIF antibody, compared with ISO-1 (30 μg) (fig. 2, E and F; n = 6). These data show that the increased level of spinal MIF is a contributor to peripheral nerve injury-induced neuropathic pain, and this role can be blocked by intrathecal administration of MIF tautomerase inhibitor that is similar in its role with MIF antibody.

**CCI Induced Up-regulation of MIF Receptor CD74**

The expression of MIF protein in the ipsilateral spinal cord dorsal horn up-regulated significantly (western blotting detection) (fig. 3A; P < 0.001; n = 6 in the sham group and n = 8 in the CCI group), and such change in expression occurred because of day 3 after the CCI surgical procedures (fig. 3B; n = 8). Recent evidence considered CD74 as a putative membrane receptor of MIF. Correspondingly, the protein expression of CD74 was up-regulated in the ipsilateral dorsal horn after CCI nerve injury (fig. 3C, and D; n = 5 in the sham group and n = 8 in the CCI group). These data display the expression of spinal MIF and its receptor, CD74, changed in a parallel manner after CCI injury and indirectly show the increased MIF exerted function through binding to the up-regulated receptor of CD74.

**Spinal Microglial Cells Are the Major Target of MIF Function in CCI Animals**

MIF is produced by nearly all types of tissues in mammals, but variant roles were played by MIF in different organic tissues. Hereby, we overall found that spinal MIF was increased after CCI Induced Up-regulation of MIF Receptor CD74

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**SCI Induced Up-regulation of MIF Receptor CD74**

The expression of MIF protein in the ipsilateral spinal cord dorsal horn up-regulated significantly (western blotting detection) (fig. 3A; P < 0.001; n = 6 in the sham group and n = 8 in the CCI group), and such change in expression occurred because of day 3 after the CCI surgical procedures (fig. 3B; n = 8). Recent evidence considered CD74 as a putative membrane receptor of MIF. Correspondingly, the protein expression of CD74 was up-regulated in the ipsilateral dorsal horn after CCI nerve injury (fig. 3C, and D; n = 5 in the sham group and n = 8 in the CCI group). These data display the expression of spinal MIF and its receptor, CD74, changed in a parallel manner after CCI injury and indirectly show the increased MIF exerted function through binding to the up-regulated receptor of CD74.
chronic sciatic nerve injury, and this increase in MIF expression takes an essential part in the regulation of the pain behaviors. However, the question of which type of cells is the primary target of MIF function in the spinal cord in this pathologic condition needs to be verified. We used CD11b, glial fibrillary acidic protein, and NeuN as markers of spinal microglia, astrocytes, and neurons, respectively, and colocalized these molecules with CD74 using immunofluorescence. As displayed in figure 3E, CD74 and CD11b presented together in the spinal microglial cells, but glial fibrillary acidic protein and NeuN were not. Therefore, it can be ascertained that the increased MIF in the spinal cord dorsal horn after CCI is mainly focused on the microglia as the primary functional target.

**ERK-1/2 (p44/p42 MAPK) Cascade Is the Downstream Signaling of Spinal MIF in CCI-treated Mice**

The expression of phosphorylated p44/p42 in the ipsilateral spinal cord dorsal horn in the CCI mice was significantly increased compared to the sham group (fig. 2A and B). This increase was dose-dependent, with the highest effect at 30 μg ISO-1 (fig. 2; n = 6 in the sham group and n = 8 in the CCI group). ISO-1 40 μg > 30 μg > 20 μg > 10 μg > DMSO = saline; *P < 0.05 saline, DMSO, or ISO-1 10 μg vs. ISO-1 10–40 μg, respectively. (C and D) Saline and the vehicle, DMSO, did not change both of the pain responses evoked by mechanical and thermal stimuli (n = 6; *P < 0.05 CCI vs. Sham; #P < 0.05 CCI/saline or CCI/DMSO vs. CCI/ISO-1 30 μg). (E and F) Intrathecal administration of anti-MIF monoclonal antibody produced a similar preventing effect as ISO-1 30 μg on pain behavioral responses (n = 6; *P < 0.05 vs. Sham; #P < 0.05 CCI/MIF Ab or CCI+ISO-1 30 μg). Data are shown as means ± SDs. All experiments were performed on the ipsilateral hind paws. MIF = macrophage migration inhibitory factor; ISO-1 = MIF tautomerase inhibitor (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid, methyl ester; CCI = chronic constriction sciatic nerve injury; DMSO = dimethyl sulfoxide; Ab = antibody.

**Spinal Neurons Are the Major Source of MIF Production after CCI Injury**

After verifying microglial cells as the functional target of increased MIF in the CCI-induced spinal cord dorsal horn, we next traced the origin of MIF using immunofluorescent colocalization, too. We found, interestingly, that MIF and NeuN appeared simultaneously in the spinal neurons, but not with CD11b and glial fibrillary acidic protein (fig. 4, A–I; n = 5). Such a change in MIF and NeuN coexisting in spinal neurons demonstrates that neurons in the spinal cord dorsal horn are the major source of MIF production after CCI injury. Thus, a possible bridge may exist between the spinal neurons and microglial cells through functionally increased MIF after peripheral nervous injury (**i.e.**, MIF produced by the spinal neurons plays a function via binding CD74 located in the microglial cells).
up-regulated, and this increase could be inhibited by an intrathecal MIF tautomerase inhibitor (detected at day 14 after CCI or sham; fig. 5A; n = 6). The expression of MIF receptor CD74 measured using western blotting was up-regulated in the ipsilateral spinal cord dorsal horn (n = 5 in the sham group and n = 8 in the CCI group; *P < 0.001; B: #P < 0.05 vs. D1, #P < 0.05 vs. D3 or D7, respectively; D: day). (C) The expression of MIF receptor CD74 measured using western blotting was up-regulated in the ipsilateral spinal cord dorsal horn (n = 5 in the sham group and n = 8 in the CCI group; *P, #P, and **P all < 0.001; D: day). (D) CD74 expression detected with immunofluorescence significantly increased in the ipsilateral dorsal horn after CCI injury (a and b; n = 6; red color: Texas Red–conjugated antibody; scale bar: 120 μm). (E) Colocalization of MIF receptor CD74 and microglia marker CD11b, astrocyte marker GFAP, and neuron marker NeuN, and found that spinal microglial cells were the functional target of MIF in CCI-treated spinal cord (a to c, a’ to c’, and a” to c”) (n = 4; red color: Texas Red–conjugated antibody; green color: FITC-conjugated antibody; scale bar: 40 μm). Data are shown as means ± SDs. CCI = chronic constriction sciatic nerve injury; MIF = macrophage migration inhibitory factor; FITC = fluorescein isothiocyanate; Ipsi = ipsilateral; Contra = contralateral; GFAP = glial fibrillary acidic protein; NeuN = neuronal nuclei; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.
tion as were phospho-p44/p42, IL-8, and NR2B (fig. 5D; n = 11005). To verify the fact of p44/p42 as the downstream cascade of spinal MIF further, we used a specific inhibitor of ERK1/2 (PD98059) intrathecally, and found that PD98059 had a similar role in reversing CCI-evoked mechanical and thermal pain behavioral responses as those of ISO-1 (30 μg) (fig. 5, E and F; n = 11005 in the sham group and n = 7 in the CCI group) and in down-regulating the expression of phospho-p44/p42 downstream effectors significantly (measured at day 14 after CCI or sham; fig. 5, G and H; n = 6). These data demonstrate that the increased spinal MIF in CCI mice exerts functions through activating the downstream signaling cascade of ERK1/2, and the phosphorylated p44/p42 leads to further production of IL-8, PGE2, and NR2B, and then these increased molecules in spinal cord finally affect the pain behaviors.

**Knockout of MIF Ameliorates Pain Perception of CCI Mice and Reduces Spinal Levels of Noxious Molecules**

We used genetically modified mice (MIF knockout) to investigate the role for MIF in the regulation of pain behavioral responses and corresponding changes in downstream molecules. The threshold of mechanical stimuli increased and the latency of thermal stimuli lengthened in the MIF−/− mice by 40–50%; besides, the decrease in the mechanical threshold and the thermal latency after nerve injury in the MIF−/− animals were smaller than the wild-type 1 s (fig. 6, A and B; n = 3 in the mechanically stimulated knockout group, n = 2 in the thermally stimulated knockout group, and n = 6 for the wild-type animal). Correspondingly, the expression of MIF receptor CD74, phospho-p44/p42, IL-8, and NR2B were down-regulated in the ipsilateral spinal cord dorsal horn of the MIF−/− CCI mice (fig. 6C; n = 3; measured at day 14 after CCI or sham). Moreover, the CSF content of PGE2 in the MIF−/− CCI mice also decreased significantly, compared with the wild-type animals (fig. 6D; n = 3 in the wild type and n = 2 in the knockout; measured at day 14 after CCI or sham). These results indicate further that spinal MIF contributes to the pathologic regulation of the pain behaviors and underlying molecules in CCI-injured mice.

*Fig. 4.* Production of MIF by the spinal neurons after CCI injury. Immunofluorescent colocalization revealed that MIF coexists with NeuN (G–I; n = 5), but not with CD11b or GFAP (A–F; n = 5) in the ipsilateral spinal cord dorsal horn after CCI injury (red color: Texas Red–conjugated antibody; green color: FITC-conjugated antibody; scale bar: 40 μm). MIF = macrophage migration inhibitory factor; CCI = chronic constriction sciatic nerve injury; FITC = fluorescein isothiocyanate; GFAP = glial fibrillary acidic protein; NeuN = neuronal nuclei.
Exogenous rMIF Produced Mimetic Effects such as the CCI Injury

The increased content of MIF in CCI-injured CSF showed that it may be a crucial component in the regulation of pain behavioral responses. To clarify this hypothesis, we intrathecally administered mouse rMIF (25 and 50 μg), and found that rMIF (50 μg) mimicked the effect of CCI-induced pain behaviors, and this effect could be reversed by the ERK1/2 inhibitor (PD98059).

**Fig. 5.** Role for ERK1/2 cascade in the spinal MIF-associated effect. The up-regulated expression of phospho-p44/p42 could be blocked by intrathecal MIF inhibitor ISO-1 (n = 5; *P < 0.01, **P < 0.05, ***P < 0.001, ††P < 0.01; detected at day 14 after CCI or sham). (A) The expression of ERK1/2 downstream effectors increased in CCI mice, and this change could be inhibited by ISO-1 (n = 5; * and **P < 0.01, ††P < 0.05; all were detected at day 14 after CCI or sham). (C) CCI-induced up-regulation of phospho-p44/p42 proteins displayed a time-dependent manner, and the total-p44/p42 proteins were vice versa (n = 6; *P < 0.05 and ‡‡P < 0.01 vs. CCI-D0-phospho; D: day). (D) CSF content of PGE2 detected with EIA kit increased in CCI mice, and such an increase could be inhibited by ISO-1 (n = 6; *P < 0.05 vs. CCI-day 3 and CCI/ISO-1-day 3, respectively; ††P < 0.01 vs. CCI-day 14 and CCI/ISO-1-day 14, respectively). (E and F) Intrathecal administration of an ERK1/2 inhibitor (PD98059) reversed both types of pain responses that was similar to the ISO-1 30 μg (n = 6 in the sham group and n = 7 in the CCI group; *P < 0.05 vs. Sham; †P < 0.05 vs. PD98059 and ISO-1). (G and H) The up-regulated expression of phospho-p44/p42, IL-8, and NR2B was blocked by intrathecal PD98059 as was the ISO-1 (n = 6; G: *P < 0.01; H: †P and ††P < 0.001 vs. CCI; detected at day 14 after CCI or sham). Data are shown as means ± SDs. ERK = extracellular signal-regulated kinase; MIF = macrophage migration inhibitory factor; ISO-1 = MIF tautomerase inhibitor (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid, methyl ester; CCI = chronic constriction sciatic nerve injury; CSF = cerebrospinal fluid; PGE2 = prostaglandin E2; EIA = enzyme immunoassay assay; IL-8 = interleukin 8; NR2B = N-methyl-D-aspartic acid receptor type 2 subunit B.
inhibitor, PD98059 (fig. 7, A and B; *P < 0.01; n = 6). Although external rMIF (50 μg) could produce such an effect on pain responses in naïve mice, the expression of IL-8 and NR2B had changed in both dosages of MIF (25 and 50 μg), and such a role produced by the externally delivered rMIF could also be blocked by PD98059 (fig. 7C; n = 6).

These data show the increased level of MIF in the CSF of the CCI mice plays a role in regulating pain behaviors and underlying noxious molecules. Therefore, MIF in the CSF as well as in the ipsilateral spinal cord dorsal horn together regulate the pain behavioral responses after peripheral nerve injury, and these effects can be reversed by the MIF tautomerase inhibitor.

**Blockade of MIF Tautomerase Activity Produced Inhibition of MIF Biologic Function**

In this study, we selected a specific inhibitor of MIF that focused on antagonizing MIF tautomerase activity. To verify the actual inhibitory role of ISO-1, a mouse microglial cell strain and CSF from CCI mice were used to testify to its inhibition on the tautomerase activity of MIF. We found MIF tautomerase activity had been inhibited by ISO-1 in a dose-dependent manner and as were the production of MIF as well in the cultured microglial cells (fig. 8, A and B; n = 6).

In addition, the tautomarization of CSF MIF from CCI-treated mice was also effectively antagonized by ISO-1, and the MIF level was decreased after ISO-1 administration (fig. 8, C and D; n = 6).

In our study, we administered mouse rMIF intrathecally to mimic CCI-induced pain behaviors in naïve mice. Therefore, the biologic and enzymatic activities of rMIF were analyzed using cultured microglial cells. External rMIF up-regulated the expression of phospho-p44/p42 and increased the production of PGE2 in a dose-dependent manner, but the activity of dopachrome tautomerization was similar in both the low and high doses of rMIF treatment (fig. 8, E-G; n = 5). These results indicate that ISO-1 and rMIF played their own roles (i.e., ISO-1 could effectively block MIF tautomerase activity and corresponding biologic function), and rMIF exerts its effect on dopachrome tautomerization and related biologic activity, too.

**Discussion**

In this study, we demonstrated that the expression levels of the proteins of MIF and MIF receptor CD74 were up-regulated in the wild-type animal (A; n = 3) and the withdrawal latency of thermal stimuli (B; n = 2) were shifted upward in MIF-/- mice that underwent CCI or sham surgery (*P < 0.05 vs. Wild-Type Sham; **P < 0.05 vs. MIF-/- CCI; ***P < 0.05 vs. MIF-/- Sham; *P < 0.05 vs. Wild-Type Sham; n = 6 for the wild-type animal). (C) Cellular changes after MIF gene knockout (i.e., the expressions of proteins CD74, phospho-p44/p42, IL-8, and NR2B) did not show significant changes in the MIF-/- mice in whichever surgeries, sham or CCI, they underwent (n = 3; *P < 0.01 vs. WT-S; **P < 0.01 vs. WT-C; WT = wild type; KT = knockout; C = sham; CCI = chronic constriction sciatic nerve injury; IL-8 = interleukin 8; NR2B = N-methyl-D-aspartic acid receptor type 2 subunit B; CSF = cerebrospinal fluid; PGE2 = prostaglandin E2.)
lated in a time-dependent manner in the spinal cord dorsal horn after peripheral nerve injury, and such changes were strongly in association with rodent neuropathic pain-like hypersensitivities that could be protected or reversed by the intrathecally administered MIF tautomerase inhibitor. Further, spinal MIF enhanced the expression of the N-methyl-D-aspartic acid receptor subunit, NR2B, and the production of IL-8 and PGE2 via ERK 1/2-related signaling cascade. Finally, MIF gene knockout and exogenous mouse rMIF alleviated and mimicked nerve injury-induced pain behavioral responses and corresponding cellular changes, respectively. These data highlight an underlying mechanism of spinal MIF, which functions as a major contributor to neuropathic pain-like hypersensitivity through activating the ERK1/2 pathway, resulting in the production of NR2B, IL-8, and PGE2.

MIF, a 12.5-kDa protein, was one of the earliest cytokines reported for its proinflammatory effects and has recently been implicated in a wide spectrum of diseases. The expression of MIF in the central nervous system and peripheral nervous fibers after nerve injury indicated that MIF plays a role in the regulation of the process of nerve degeneration/regeneration. In addition to the ascertained relation between nerve injury and pain, we tested the role of MIF in formalin-induced inflammatory pain, and found that MIF is involved in pain regulation in rats. Given the distinctively different features between neuropathic pain and inflammatory or acute pain, we then hypothesized that spinal MIF might also be involved in the regulation of nerve injury-evoked neuropathic pain. We used a mouse model of neuropathic pain and proved the increased level of MIF in CSF and the up-regulated expression of MIF in the ipsilateral spinal cord dorsal horn were strongly associated with CCI-induced pain behaviors. An interesting difference to note was the MIF production in CSF and the spinal cord (i.e., inflammatory pain induced by formalin injection resulted in a faster production of MIF), during which a statistically significant increase was observed at 30 min in CSF and 5 min in the spinal cord after stimuli, but neuropathic pain induced by nerve injury displayed a relatively delayed production of MIF beginning on day 3 after CCI. We found in our previous study and in the current one that spinal microglial cells and neurons may be the major source of MIF in the inflammatory and neuropathic contexts, respectively. The different time windows of MIF production in these two pain states suggest there may exist distinctive functional connections among MIF, microglial cells, and neurons in inflammatory and neuropathic pain conditions.

Fig. 7. Effect of rMIF on pain behaviors and cellular changes in naïve mice. External rMIF (50 μg) administrated intrathecally produced a mimetic effect on both kinds of pain stimuli on day 7, and this effect could be inhibited by coadministration of ERK1/2 inhibitor (A and B; n = 6; *p < 0.01). (C) The expressions of IL-8 and NR2B in rMIF-treated naïve mice were significantly up-regulated in both rMIF dosages of 25 and 50 μg, and these changes could be blocked by ERK1/2 inhibitor (n = 6; *p < 0.05 vs. V in IL-8 expression; **p < 0.01 vs. V in NR2B expression). Data are shown as means ± SDs. V = vehicle; D = day; M25 = rMIF 25 μg; P = PD98059 (ERK1/2 inhibitor). rMIF = recombinant macrophage migration inhibitory factor; ERK = extracellular signal-regulated kinase; IL-8 = interleukin 8; NR2B = N-methyl-D-aspartic acid receptor type 2 subunit B.
The expression of CD74, the putative transmembrane receptor of MIF, was up-regulated in the ipsilateral spinal cord dorsal horn after CCI indirectly showed that the spinal cord, especially the spinal microglial cells, were the initial locus of MIF function in the regulation of neuropathic pain and downstream cellular respondents because of the following reasons: first, CD74 level increased in the spinal cord (immunofluorescence) and colocalized with CD11b in microglial cells; second, MIF-CD74 downstream signaling ERK1/2 was activated by peripheral nerve injury; third, the expression of proteins of ERK1/2-associated effectors (IL-8, NR2B, and PGE2) could be inhibited by the MIF inhibitor; and finally, MIF gene mutation desensitized pain perception and reduced CD74 and downstream cellular effectors.

The activation of the ERK1/2-p44/p42 MAPK cascade has been guaranteed and shown to be responsible for MIF action by binding to CD74.32 The increased content of phospho-p44/p42, as well as NR2B, after CCI in the ipsilateral spinal cord dorsal horn was consistent with previous reports regarding ERK1/2 activation and N-methyl-D-aspartic acid receptor up-regulation.31,52 Besides, ERK1/2 downstream effectors, such as IL-8 and PGE2, also displayed corresponding changes along with MIF-activated ERK1/2 phosphorylation in CCI mice, and these changes could be blocked by spinal PD98059, a specific antagonist of ERK1/2, or abolished and mimicked by MIF gene knockout and exogenous rMIF administration, respectively. The production of IL-8 in CSF after spinal cord injury has been suggested as a factor to stratify injury severity and predict neurologic outcome.11 Although IL-8 is not involved in the development of heat hyperalgesia in rat skin models,53 it contributes to the pathophysiology of pain in length-dependent, small-fiber neuropathy54 and inflammatory pain.55 In our study, the expression of IL-8 in the ipsilateral spinal cord dorsal horn after CCI was critically up-regulated; this indicates further studies are needed to warrant the precise role of spinal IL-8 in neuropathic pain. PGE2 itself has been shown to be associated with the regulation of neuropathic pain,56 but this effect was generally attributed to the upstream molecule of cyclooxygenase 2. In our study, we measured PGE2 as the effector of ERK1/2 activation, but the relation with cyclooxygenase 2 was not investigated. Although MIF could up-regulate cyclooxygenase 2 expression and PGE2 production in other pathologic conditions,57 the possible relation of MIF and cyclooxygenase 2 in neuropathic pain is yet to be guaranteed. Our study, at least in part, demonstrates that...
spinal MIF-CD74-ERK1/2-NG-methyl-d-aspartic acid receptor and/or PGE2 pathways are activated after peripheral nerve injury in rodents. While such conclusions can be drawn from the current study, we cannot guarantee its generalizability with other types of neuropathic pain models.

Our previous work has shown that ERK/p38 MAPK was activated by inflammatory stimuli31; however, the current study received a different result that ERK1/2 (p44/p42 MAPK) was the responding signaling in rodent neuropathic pain-like hypersensitivity induced by peripheral nerve injury. In inflammatory pain conditions, spinal microglial cells were identified as the major source of MIF. However, in CCI-induced neuropathic pain, we found spinal neurons are the major source of MIF production, but microglial cells are the primary functional target of MIF detected with CD74 and CD11b colocalization. These interesting findings reveal a functional communication between spinal neurons and microglial cells bridging by proinflammatory cytokine MIF. Although recently published literature suggested glial-derived molecules within the dorsal horn of the spinal cord modulate pain signaling and contribute to self-perpetuating pain,58 microglia-neuron interaction through pronociceptive mediators, including inflammatory cytokines, appears to play more important roles in driving chronic neuropathology and/or chronically primed conditions that accounts for their contribution to neuropathic pain.59 Our data figured out a signaling circuit between spinal neurons and microglial cells displaying through MIF production by neurons that consequently exert function by binding to CD74 located in microglial cells, which seems, in turn, to regulate the neuronal perception from peripheral nervous injury. Such changes caused by microglia-neuron cross talk may account for the behavioral responses of neuropathic pain induced by CCI injury. In addition, the diverse results of the current study from the previously reported inflammatory pain studies31 demonstrate that spinal MIF with different origins, though, plays a role in affecting inflammatory and neuropathic pain behaviors that different signaling transduction pathways are involved in.

The MIF tautomerase inhibitor was used intrathecally in this study to investigate the role of MIF in pain regulation, which was mainly based on the fact that the blockade of tautomization could inhibit MIF biologic activities.20,33 Consistently, spinal use of ISO-1 effectively attenuated CCI-induced pain behavioral responses and cellular changes that were identical to the effect of spinal MIF antibody. The intrathecal administration of drugs is an effective, promising method in pain study,34–36 but it is difficult to perform in clinical contexts to general physicians. So, that drugs used in a nonintrathecal means could reach the central nervous system by passing the blood-cerebrospinal fluid barrier easily was considered as one attractive routine, but this mainly relies on the physical and chemical properties of the drugs, such as molecular weight, molecular diameter, hydrophilicity, lipid solubility, and pKa. The results of our study showed spinal MIF could be inhibited by intrathecal MIF tautomerase inhibitor ISO-1, and indicated that it might be a unique target of pharmacological treatment. So, the designing of drugs focused on MIF tautomization that could pass through the blood-cerebrospinal fluid barrier possesses pivotal clinical and pharmacological implications.33

Neuroinflammation is an essential part contributing to the onset and development of pain through neural-immune interactions that activate immune cells, glial cells, and neurons, leading to the debilitating pain state.60 Although persistent or recurring exposure of neurons to activated immune cells is related with an increase in painful behaviors, the knowledge of the functional consequences of immune cell-neuron interaction is still incomplete. Our previous findings of MIF in inflammatory pain,31 combined with the role for MIF in CCI-induced neuropathic pain conditions in the current study, has extended our understanding of the inflammatory responses in the regulation of pain. Neuroinflammation resulting from the activated spinal neurons, the major source of MIF production, and the microglial cells, the primary target of MIF function, after peripheral nerve injury has directly linked these two types of cells functionally together in the spinal cord by the shuttled MIF between them. The increased MIF consequently resulted in downstream activation of proinflammatory mediators IL-8 and PGE2, which, in turn, account for the development of neural inflammation leading to changes in nociceptive behaviors. Besides, MIF caused an up-regulation of downstream NR2B expression, one subtype of the ionotropic glutamate receptors known to be critical for triggering long-lasting changes in synaptic plasticity.61 These changes in MIF-p44/p42-NR2B/IL-8/PGE2 cascade advance our understanding of neuroinflammation in supporting nociception after peripheral nerve injury. On the other hand, it is hopeful to develop pharmacotherapy for the management of neuropathic pain and restore the beneficial alarm function of pain by stabilizing the hyperactivated microglia-neuron communication using anti-MIF agents, MIF receptor antibodies, MIF-signaling inhibitors, or microglial and neuronal stabilizers.62 However, the precise mechanisms underlying such microglia-neuron communication through proinflammatory cytokine is not well known and needs to be investigated further.

Activation of ERK1/2 in the spinal cord is strongly associated with the generation and development of different types of pain conditions, including bee venom-induced inflammation and hyperalgesia,63 complete Freund adjuvant-produced peripheral inflammation, mechanical allodynia, and thermal hyperalgesia,64 and persistent mechanical hyperalgesia in ovicertomized mice.65 Of the two isoforms of ERK1/2, ERK1 plays a limited role in nociceptive sensitization, but ERK2 has a predominant role in murine pain models.66 In the spinal cord, the activated ERK1/2 can induce c-Fos expression and trigger the transcription of prodynorphin, which results in a long-lasting increase in dynorphin level that participates in the persistence of pain.67 Besides, the peripheral ERK1/2 pathway in the primary injury site is also required to maintain a melittin-enhanced wind-up of rat spinal cord wide-dynamic-range neurons, a pro-
cess of peripheral modulation of wind-up enhancement in nociceptive conditions.68 Our study has testified to the role for ERK1/2 as a downstream signaling effector of the activated spinal MIF in peripheral nerve injury-induced neuropathic pain-like hypersensitivity through inducing further production of NR2B, IL-8, and PGE$_2$, which is a support to previous reports like hypersensitivity through inducing further production of nal MIF in peripheral nerve injury-induced neuropathic pain.

Based on the observation of increased levels of MIF in the CCI injury-induced CSF, we intrathecally administered rMIF to naïve animals to determine whether or not the exogenous MIF could mimic peripheral nerve injury-induced changes in nociceptive behaviors and cellular molecules. Although intrathecal injection of rMIF caused sensitization to mechanical and thermal stimuli and up-regulation of IL-8 and NR2B expression, we still cannot exclude the possibility of CSF MIF functioning separately from the MIF produced within the spinal tissue locally, or that they play roles synergically. In the current study, microglial cells were found to be the functional site of locally produced MIF in the spinal cord dorsal horn, but how the CSF MIF exerting its function alone or in concert is not well investigated. With the fact that intrathecal administration of the small-molecule inhibitor of MIF prevented pain behavioral and cellular changes plus findings in MIF gene-modified animals, we can conclude, in part, that CSF MIF and tissue MIF function separately first, and then together, which should be confirmed by future studies.

Here, we, to our knowledge, are the first to identify spinal MIF as a major contributor to sciatic nerve injury-induced neuropathic pain-like hypersensitivity. We provide evidence that (i) intrathecal MIF inhibitor protected or reversed CCI-induced pain behaviors that were similar as was anti-MIF antibody; (ii) spinal MIF evoked activation of ERK1/2 signaling cascade by binding its transmembrane receptor, CD74; (iii) the expression of N-methyl-D-aspartic acid receptor was up-regulated after CCI, which could be blocked by the spinal MIF inhibitor; (iv) MIF gene mutation and exogenous rMIF could desensitize and mimic CCI-evoked pain responses and cellular changes, respectively; and (v) MIF-specific tautomerase inhibitor ISO-1 could effectively antagonize the biologic activities of MIF. Hence, spinal MIF may be a potential therapeutic target by inhibiting MIF-activated ERK1/2-N-methyl-D-aspartic acid receptor and/or-PGE$_2$ cascade during peripheral nerve injury induced neuropathic pain.

References


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ANESTHESIOLOGY REFLECTIONS

Gwathmey’s “Expansible” Then “Expanding” Airway

Filed originally as an “Expansible Respiratory Airway” (left) on March 27, 1937, U.S. Patent No. 2,127,215 was granted on August 16, 1938, to James T. Gwathmey, M.D. (1862–1944), of New York. Renaming it an “Expanding Airway Tube” less than a year later, Gwathmey observed that after “insertion, the blades are opened as one would manipulate a vaginal speculum, by turning a small screw . . . making possible natural breathing (nasal and oral).” Photographed in mirror image (right) for direct comparison to Gwathmey’s earlier depictions of his airway, the Wood Library Museum’s prototype bore a tag from the donor explaining that “This was made by Dr. James T. Gwathmey and was given to me on the occasion of his visit here.—J. S. Lundy, Mayo Clinic, Rochester, Minnesota.” (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

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