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Jmjd3 mediates blood–spinal cord barrier disruption after spinal cord injury by regulating MMP-3 and MMP-9 expressions

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ABSTRACT

The disruption of the blood–spinal cord barrier (BSCB) by matrix metalloprotease (MMP) activation is a detrimental event that leads to blood cell infiltration, inflammation, and apoptosis, thereby contributing to permanent neurological disability after spinal cord injury (SCI). However, the molecular mechanisms underlying *Mmp* gene regulation have not been fully elucidated. Here, we demonstrated the critical role of histone H3K27 demethylase Jmjd3 in the regulation of *Mmp* gene expression and BSCB disruption using *in vitro* cellular and *in vivo* animal models. We found that Jmjd3 up-regulation, in cooperation with NF-κB, after SCI is required for *Mmp*-3 and *Mmp*-9 gene expressions in injured vascular endothelial cells. In addition, Jmjd3 mRNA depletion inhibited *Mmp-3* and *Mmp-9* gene expressions and significantly attenuated BSCB permeability and the loss of tight junction proteins. These events further led to improved functional recovery, along with decreased hemorrhage, blood cell infiltration, inflammation, and cell death of neurons and oligodendrocytes after SCI. Thus, our findings suggest that Jmjd3 regulation may serve as a potential therapeutic intervention for preserving BSCB integrity following SCI.

1. Introduction

The blood–spinal cord barrier (BSCB) and blood–brain barrier (BBB) are highly selective permeable vascular endothelial structures in the central nervous system (CNS). The BSCB and BBB primarily consist of vascular endothelial cells that are tightly associated by well-developed tight junctions (TJs), which regulate the restriction of the entry of plasma components and blood cells into the brain or the spinal cord (Drewes, 2001; Hawkins and Davis, 2005). However, BBB/BSCB disruption following traumatic CNS injury generates neurotoxic products that impair synaptic and neuronal functions (Hawkins and Thomas, 2004; Abbott et al., 2006; Zlokovic, 2008). BBB/BSCB disruption further induces the programmed death of neurons and glia, resulting in permanent neurological deficits (Xu et al., 2001; Noble et al., 2002; Gerzanich et al., 2009). Therefore, the prevention of BSCB disruption should be considered as a potential therapeutic intervention after spinal cord injury (SCI).

Matrix metalloproteases (MMPs), a family of zinc endopeptidases, are known to degrade extracellular matrix and to process cytokines and growth factors (Sternlicht et al., 1999; Sternlicht and Werb, 2001). Thus, MMPs play an important role in diverse biological processes, including animal development and wound healing. However, the aberrantly excessive activity of MMPs exerts harmful effects or leads to numerous pathological conditions such as BSCB/BBB disruption after CNS injury (Rosenberg et al., 1994, 1995, 1998; Xu et al., 2001; Asahi et al., 2001; Noble et al., 2002). For example, MMP-9 induces the proteolytic degradation of BBB and white matter (WM) components, such as myelin basic protein, leading to an increase in the infarct volume after transient cerebral ischemia (Asahi et al., 2001). Up-regulated MMP-9 is further involved in abnormal vascular permeability and inflammation in the early stage after SCI (Lee et al., 2012b, 2012c) Consistently, blocking of MMP-9 activity improves functional recovery due to the restoration of vascular permeability (Noble et al., 2002). In addition, BSCB permeability and microglia/macrophage activation were reduced in *Mmp-12* KO mice after SCI, resulting in significantly improved functional recovery (Wells et al., 2003). Recently, we also reported that MMP-3 is involved in SCI-induced BSCB disruption (Lee et al., 2014a).

Abbreviations: AAV, adeno-associated virus; BBB, blood–brain barrier; CNS, central nervous system; ChIP, Chromatin Immunoprecipitation; Cox, cyclooxygenase; GM, gray matter; HDAC, histone deacetylase; MMP, matrix metalloprotease; OGD, oxygen–glucose deprivation;; shRNA, short hairpin RNA; SCI, spinal cord injury; BSCB, blood–spinal cord barrier; TJ, tight junction; VPA, valproic acid; WM, white matter.

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Several studies have reported the effects of histone modifications or histone- modifying enzymes on pathological processes after SCI. For instance, the expression of the histone acetyltransferase p300 is increased in the lumbar spinal cord and its inhibition reversed injury-induced mechanical allodynia and thermal hyperalgesia via the suppression of brain-derived neurotrophic factor and cyclooxygenase (*Cox)-2* gene expression upon chronic constriction injury (Zhu et al., 2012, 2014). However, most studies have focused on the function of histone deacetylase (HDAC) inhibitors as therapeutic agents for SCI. Valproic acid (VPA) treatment attenuates apoptosis and promotes locomotion recovery after SCI via the up-regulation of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor (Lv et al., 2011, 2012). VPA also decreases the up-regulation of MMP-9 expression after SCI, results in the attenuation of BSCB disruption and an improvement in functional recovery (Lee et al., 2012b). Moreover, VPA-treated rats showed decreased accumulation of microglia/macrophages and astrocytes in the injured spinal cord (Lu et al., 2013). VPA treatment induces the over-expression of microtubule-associated protein 2 (MAP2), leading to the promotion of neurite outgrowth in rats after SCI (Finelli et al., 2013; Abdanipour et al., 2015). Neuropathic pain is also significantly reduced by MS-275 treatment after SCI (Denk et al., 2013). Recently, we reported that the acute up-regulation of the histone H3K27me3 demethylase Jmjd3 is required for *IL-6* gene activation in injured blood vessels after SCI (Lee et al., 2012d).

In this study, we examined whether Jmjd3 plays an important role in BSCB integrity after SCI using *in vitro* cellular and *in vivo* animal models. We found that Jmjd3 activates MMPs gene expression in injured blood vessels after SCI and in vascular endothelial cells after oxygen–glucose deprivation (OGD)/reperfusion injury. In addition, *Jmjd3* mRNA depletion resulted in reduced MMPs expression and BSCB disruption, leading to improved functional recovery, along with decreased hemorrhage, blood infiltration, inflammation, and apoptosis of neurons and oligodendrocytes, after SCI.

2. Material and methods

2.1. Spinal cord injury

Adult rats [Sprague–Dawley; male; 250–270 g; Sam:TacN (SD) BR, Samtako, Osan, Korea] were anesthetized with chloral hydrate (500 mg/kg) and a laminectomy was performed at the T9–T10 level exposing the cord beneath without disrupting the dura. The exposed dorsal surface of the spinal cord then was subjected to moderate contusion injury (25 g-cm) using a NYU impactor as previously described (Lee et al., 2010). For the sham-operated controls, animals underwent a T10 laminectomy without weight-drop injury. All surgical interventions and postoperative animal care were performed in accordance with the Guidelines and Polices for Rodent Survival Surgery provided by the Animal Care Committee of the Kyung Hee University.

2.2. Tissue preparation

Tissue preparation was performed as previously described (Lee et al., 2010). At indicated time points after SCI, animals were anesthetized with chloral hydrate (500 mg/kg) and perfused via cardiac puncture initially with 0.1 M PBS and subsequently with 4% paraformaldehyde in 0.1 M PBS. A 20-mm section of the spinal cord, centered at the lesion site, was dissected out, post-fixed by immersion in the same fixative for 5 h and placed in 30% sucrose in 0.1 M PBS. The segment was embedded in OCT for frozen sections, and longitudinal or transverse sections were then cut at 10 or 20 μm on a cryostat (CM1850; Leica, Germany). For molecular work, animals were perfused with 0.1 M PBS and segments of spinal cord (1 cm) including the lesion site were isolated and frozen at −80 °C.

2.3. Endothelial cell culture and OGD/reperfusion

Mouse brain microvessel endothelial cell line bEnd.3 (ATCC, Menassas, VA) maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics. To achieve OGD, cultures were transferred to a humidified anaerobic chamber (APM-30D, Astec, Fukuoka, Japan) under an atmosphere of 5% CO_2 balanced with 95% N_2. The culture medium was replaced three times with deoxygenated and glucose free OGD buffer (116 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.4 mM KCl, 1 mM NaH_2PO_4, 14.7 mM $NaHCO_3$, 10 mM HEPES, pH 7.4). <1% oxygen and a temperature of 37 °C were maintained throughout the OGD period. At the end of the OGD period, all cultures were placed under normoxic conditions and the media was quickly replaced with glucose-containing DMEM as previously described (Lee et al., 2012d).

2.4. RNA isolation and PCR analysis

Total RNA was extracted from spinal cord tissues and cells using TRIZOL reagent (Invitrogen, Carlsbad, CA) or RNeasy mini kit (Qiagen, Valencia, CA). Quantitative PCR was performed using a Stratagene Mx3000P (Agilent Technologies, Waldbronn, Germany) as previously described (Lee et al., 2012d). Amplification was performed by using the following primers: 5′-ACC CCT TCA CGG GAA GTT G-3′ and 5′-TCA CTG TCG TGC TCT GAT TCA-3′ for mouse *Jmjd3*, 5′-TTC TTT GGT GCT GAG GGA CC-3′ and 5′-GGA GGG GTA TGC GAA TCC TG-3′ for rat *Jmjd3*, 5′-GGA CAA GTG GTC CGC GTA AA-3′ and 5′-CCG ACC GTT GAA CAG GAA GG-3′ for mouse *Mmp-2*, 5′-TGG AGA TGC TCA CTT TGA CG-3′ and 5′-GCC TTG GCT GAG TGG TAG AG-3′ for mouse *Mmp-3*, 5′-GTG GGA AGC CAG TGG AAA TGA TGA-3′ and 5′-CCA TGC AAT GGG TAG GAT GAG-3′ for rat *Mmp-3*, 5′-TGT CTG GAG ATT CGA CTT GAA GTC-3′ and 5′-TGA GTT CCA GGG CAC ACC A-3′ for mouse *Mmp-9*, 5′-CTT CGA AGG CGA CCT CAA GTG-3′ and 5′-TTC GGT GTA GCT TTG GAT CCA-3′ for rat *Mmp-9*, 5′-AGG TCG GTG TGA ACG GAT TTG-3′ and 5′-TGT AGA CCA TGT AGT TGA GGT CA-3′ for mouse *Gapdh*, 5′-TCA CCA TCT TCC AGG AGC GAG A-3′ and 5′-AGA CGC CAG TAG ACT CCA CGA C-3′ for rat *Gapdh*. For some *in vivo* sample, RT-PCR was performed as previously described (Lee et al., 2012b). Amplification was performed by using the following primers: 5′-CTA CAC CCA GCA TTT ATT TGG AG-3′ and 5′-GCC TAA GTT GAG CCG AAG TG-3′ for rat *Jmjd3*, 5′-ACC ATC GCC CAT CAT CAA GT-3′ and 5′-CGA GCA AAA GCA TCA TCC AC-3′ for rat *Mmp-2*, 5′- GGC TTC AGT ACC TTC CCA GG-3′ and 5′- GCA GCA ACC AGG AAT AGG TT-3′ for rat *Mmp-3*, 5′-AAA GGT CGC TCG GAT GGT TA-3′ and 5′-AGG ATT GTC TAC TGG AGT CGA-3′ for rat *Mmp-9*, 5′- GCA GCT ACC TAT GTC TTG CCC GTG-3′ and 5′- GTC GTT GCT TGT CTC TCC TTG TA-3′ for rat *IL-1β*, 5′- CCC AGA CCC TCA CAC TCA GAT-3′ and 5′- TTG TCC CTT GAA GAG AAC CTG-3′ for rat *Tnf-α*, 5′- CCA TGT CAA AAC CGT GGT GAA TG-3′ and 5′- ATG GGA GTT GGG CAG TCA TCA G-3′ for rat *Cox-2*, 5′- CTC CAT GAC TCT CAG CAC AGA G-3′ and 5′- GCA CCG AAG ATA TCC TCA TGA T-3′ for rat *iNOS*, and 5′- TCC CTC AAG ATT GTC AGC AA-3′ and 5′- AGA TCC ACA ACG GAT ACA TT-3′ for rat *Gapdh*. Quantification of bands was performed by AlphaImager software (Alpha Innotech Corporation, San Leandro, CA).

2.5. RNA interference by siRNA or shRNA

bEnd.3 cells were transfected with siRNA against mouse *Jmjd3* (M-063799-01-0005, Dharmacon, Lafayette, CO), mouse *NF-κB p50* (M-047764-1-0005, Dharmacon), mouse *NF-κB p65* (M-040776-01-0005, Dharmacon), and control siRNA (sc37007, Santa Cruz Biotechnology, Santa Cruz, CA) as previously described (Lee et al., 2012d). For the depletion of *Jmjd3* mRNA in spinal cord, *Jmjd3* siRNA (M-084605-01-0005, Dharmacon) or control siRNA (D-001206-13-05, Dharmacon) was dissolved in distilled water and injected bilaterally (2 μl/site, final 0.03 nmol per cord) into the spinal cord at 30 min after SCI as previous report (Lee et al., 2014a). Intraspinal injection into the spinal cord was performed using a pulled glass capillary pipette (30 μm external tip