Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by selective loss of upper and lower motor neurons. While the majority of ALS cases are sporadic, 5% – 10% are familial ALS, among which approximately 20% are linked to mutation in the gene encoding copper-zinc superoxide dismutase (SOD1) (1). Based on knowledge of inherited ALS in humans, a variety of mouse strains have been generated. Among these, the most studied is the G93A mouse, which harbors a point mutation in the human gene encoding SOD1, leading to a 93 glycine/alanine substitution. Overexpression of G93A causes a progressive motor neuron disease resembling most of the clinical features of human ALS, making the G93A mouse suitable for studying the pathogenesis of ALS (2). Although the cause and pathogenesis of ALS still remain largely unknown, inflammation of the lumbar spinal cord has recently been highlighted as an important pathogenic mechanism. Increasing evidence indicates that activated microglia (3), macrophages (3), and reactive astrocytes (4) are present and that they are increased in the spinal cord of ALS patients and model mice.

Prostaglandin E2 (PGE2) is the most versatile prostaglandin and activates four specific G protein–coupled receptors (GPCRs), E prostanoid (EP)-receptor subtypes 1 through 4 (5). These receptors have divergent downstream signaling cascades, cellular expression patterns, and functional effects depending on the physiological or pathological context (6, 7). There is growing evidence that PGE2 and the arachidonic acid pathway play a pivotal role in the pathogenesis of ALS. For instance, in the G93A mice, there is a marked increase of PGE2 content in both the cerebral cortex and spinal cord (8). Moreover, PGE2 levels are increased in postmortem brain tissue, cerebrospinal fluid, and serum from patients with spo-

Expression of Microsomal Prostaglandin E Synthase-1 in the Spinal Cord in a Transgenic Mouse Model of Amyotrophic Lateral Sclerosis

Hiroko Miyagishi1,†, Yasuhiro Kosuge1,†, Kumiko Ishige1, and Yoshihisa Ito1,*

1Laboratory of Pharmacology, School of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

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Abstract. Prostaglandin E2 (PGE2) is a key molecule involved in the neuroinflammatory processes that characterize amyotrophic lateral sclerosis (ALS). Although PGE2 synthesis is regulated by PGE2 synthases (PGESs), the pathological role of PGESs in ALS still remains unknown. Experiments were performed to elucidate the expression of PGESs and the localization of microsomal PGES-1 (mPGES-1) in neurons and glial cells in the spinal cord of ALS model (G93A) mice. Neurological symptom was observed in G93A mice from 14 weeks by the tail suspension test, and rotarod performances were decreased at 16 weeks and older. Western blotting revealed that the level of mPGES-1 was increased in G93A mice at 15 weeks and older. In contrast, the levels of cytosolic PGES and mPGES-2 did not change at any age. Immunohistochemical analysis demonstrated that age-dependent expression of mPGES-1 was found in motor neurons in G93A mice at 11 and 15 weeks. Immunoreactivity of mPGES-1 was also co-localized in Iba1-positive microglia in G93A mice at 15 weeks. These results suggest that mPGES-1 in motor neurons may play a role in the pathogenesis of ALS and that mPGES-1 may work sequentially in motor neurons and activated microglia to produce ALS symptoms in G93A mice.

Keywords: amyotrophic lateral sclerosis, motor neuron, microglia, prostaglandin E2, microsomal prostaglandin E synthase-1
radic ALS (9, 10). PGE₂ is sequentially synthesized from arachidonic acid by cyclooxygenases (COX-1 and COX-2) and PGE₂ synthases (PGESs). Three major isoforms of PGES have been isolated: cytosolic PGES (cPGES) and microsomal PGES (mPGES)-1 and mPGES-2 (11–13). While cPGES and mPGES-2 are constitutively expressed in various cells and tissues, mPGES-1 is induced by proinflammatory stimuli and is functionally coupled to COX-2 (11, 14). The expression of COX-2 but not COX-1 has been shown to be up-regulated in the spinal cord of ALS patients and ALS model mice (15, 16). Treatment with a selective COX-2 inhibitor, celecoxib, markedly inhibits production of PGE₂ in the spinal cords of ALS model mice (17). Furthermore, celecoxib delays onset of the disease, prolongs survival, and protects against motor neuron degeneration (17). Similarly, COX-2 inhibition by nimesulide, a less selective inhibitor than celecoxib, or rofecoxib, a more highly-selective inhibitor than celecoxib, prevented the elevation of PGE₂ content in the spinal cord of G93A mice, and resulted in a significant delay in the onset of impairment of motor activity (8, 18). It has also been reported that cytosolic phospholipase A₂ (cPLA₂) and COX-2 play an integrative role in G93A mice, and treatment with COX inhibitors as anti-inflammatory compounds is an effective strategy for delaying the onset of ALS (19). These results suggest that COX-2 and PGE₂ play an important role in the pathogenesis of ALS. Relatively recent reports demonstrated that mPGES-1 expression is not strictly coupled to the expression of COX-2 in activated primary rat microglia (20) and breast cancer (21). Nonetheless, the profiles and roles of PGESs in the spinal cord in ALS have not yet been established. In the present study, we sought to elucidate the pathogenetic roles of PGESs in neurons and glial cells in the spinal cord of ALS model mice.

Materials and Methods

Animals

Transgenic mice of the B6SJL-TgN (SOD1-G93A) 1Gur strain (Jackson Laboratory, Bar Harbor, ME, USA) were used as an in vivo model of ALS. G93A and wild-type (WT) mice were housed under standard conditions (temperature 22°C, relative humidity of 60%, 12-h light/dark cycle, and free access to food and water) in the animal facility at the School of Pharmacy, Nihon University. Genotyping was performed using genomic DNA extracted from tails by the polymerase chain reaction (PCR) as reported previously (22). We previously reported that no gender differences were observed in survival between males and females in these G93A mice (male: 151.5 ± 9.2 days vs. female: 158.3 ± 3.5 days, P = 0.66) (23). Therefore, male and female mice were combined as a group and were used in the following studies. In the present study, we used a total of 98 mice, either G93A or WT mice, allocating them to the following two groups: 12 mice of 98 were used for behavior analyses (n = 6, each) and the remaining mice were used for biochemical and histological studies. For western blotting analysis, we used 9-, 11-, 13-, 15-, 17-, 19-week-old G93A mice and age-matched WT (5 mice per week, 60 mice in total). In the histological analysis, 10 mice at the end stage of disease (19 week, n = 5, each) were used for cresyl violet-staining, and 11- and 15-week-old mice G93A and age-matched WT (4 mice per week, 16 mice in total) were used for immunohistochemistry. All efforts were made to minimize the number of animals used and their distress. All experiments with animals complied with the Guidelines for Animal Experiments at Nihon University.

Tail suspension test

The mouse was suspended by its tail and extension of hind limbs was observed as reported previously (24). The deficits score are as follows: grade 0, normal; grade 1, partial hind-limb extension; grade 2, no hind-limb extension. However, partial dysfunction of their hind limbs was not observed in G93A and WT mice at any age.

Rotarod performance test

Rotarod performance was investigated using a Rotarod apparatus (Muromachi Kikai, Tokyo). After a training period of several days, mice were able to stay on the rotarod at 24 r.p.m. Each mouse was given five trials of a maximum of 60 s and the average time on the rotarod was used as a measure for motor performance.

Western blotting

Western blots were performed as reported previously (25). Spinal cord tissue obtained from G93A and WT mice (aged 9 to 19 weeks) were homogenized in radioimmunoprecipitation assay (RIPA) buffer containing 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM Tris-HCl (pH 8.0), 1% Triton, and 5 mM EDTA; centrifuged; and then the supernatants were isolated and used. Protein concentrations were determined using the method of Bradford (26). Protein extracts were analyzed by SDS-polyacrylamide gel electrophoresis, followed by transfer of the proteins to polyvinylidene difluoride paper (Millipore). The membranes were blocked in blocking buffer containing 20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.05% Tween-20, and 5% skim milk for 1 h at room temperature and incubated with anti-non-phosphorylated neurofilament (SMI32) antibody (Sternberger Monoclonal, Inc., Baltimore, MA, USA; diluted 1:1000),
anti-Iba1 antibody (Wako, Tokyo; diluted 1:500), anti-glial fibrillary acidic protein (GFAP) antibody (Millipore, diluted 1:10,000), anti-mPGES-1 antibody (Cayman Chemical Co., Ann Arbor, MI, USA; diluted 1:500), anti-mPGES-2 antibody (Cayman Chemical Co., diluted 1:2000) or anti-cPGES antibody (Cayman Chemical Co., diluted 1:1000) overnight at 4°C. The membranes were washed repeatedly in Tris-buffered saline (20 mM Tris–HCl pH 7.6, 137 mM NaCl) containing 0.05% Tween-20, and then horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted 1:10,000) was added for 1 h. Immunoreactive bands were detected by an enhanced chemiluminescence (ECL) detection system (GE Healthcare, Chalfont St. Giles, UK). Optical density on the blots was measured with Scion imaging software (www.scioncorp.com). Quantitative results were expressed as the ratio of the band intensity of the protein of interest to the band intensity of β-actin.

Histological analysis
Mice were anesthetized with an intraperitoneal injection of 0.1 mL of pentobarbital (50 mg/mL). Anesthetized animals were perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). The lumbar spinal cord was removed and post fixed for 24 h in the same fixative. Post-fixed spinal cord was embedded in paraffin and sliced on a microtome at a 10-μm thickness. The paraffin-embedded spinal cord sections were stained with Cresyl Violet (Sigma-Aldrich, St. Louis, MO, USA). We examined the section at 20 × magnification in the anterior gray matter (left or right) for counting the number of neurons.

Immunohistochemistry
For immunohistochemistry, post-fixed lumbar spinal cord was immersed for 24 h in PBS containing 30% sucrose and horizontally sectioned on a cryostat at 10-μm thickness. The sections were permeabilized with 0.3% Triton X / PBS overnight. After blocking nonspecific binding incubated with 1.5% normal donkey serum or 1.5% normal goat serum in 0.1% Triton X / PBS, the sections were incubated with anti-mPGES-1 antibody (diluted 1:250), and anti Iba1-antibody (diluted 1:500) or anti-GFAP antibody (diluted 1:2000) for 72 h at 4°C. After washing with PBS, the sections were incubated for 2 h with Alexa Fluor 647–conjugated donkey IgG antibody, Alexa Fluor 488–conjugated donkey IgG antibody, Alexa Fluor 488–conjugated goat IgG antibody (Invitrogen, San Diego, CA, USA), or anti-NeuN Alexa Fluor 488–conjugated antibody (Millipore, diluted 1:100). After rinsing with PBS, the sections were analyzed using a confocal laser microscope (LSM-510; Zeiss, Oberkochen, Germany). Semi-quantitative analysis of change in mPGES-1 immunoreactivity was performed as reported previously (27). Motor neurons were defined according to the following three criteria: i) NeuN-positive cell; ii) localization in ventral horns; iii) diameter > 25 μm.

Statistics
All data were expressed as the mean ± S.E.M. Motor performance was analyzed with two-way repeated measures analysis of variance (ANOVA, with ‘genotype’ and ‘weeks of age’ as between-subjects factors) followed by Bonferroni’s post hoc test. Expression levels of protein were analyzed using two-way ANOVA (with ‘genotype’ and ‘weeks of age’ as between-subjects factors) followed by Bonferroni’s post hoc test. Quantification of motor neuron number in lumbosacral spinal cord was compared using a Student’s t-test. Semi-quantitative analysis of change in mPGES-1 immunoreactivity in motor neuron among all four groups (11 and 15 weeks spinal cords from WT and G93A groups, respectively) was tested with two-way ANOVA followed (with ‘genotype’ and ‘weeks of age’ as between-subjects factors) by Bonferroni’s post hoc test. Semi-quantitative analysis of change in mPGES-1 immunoreactivity in activated microglia at 11 and 15 weeks spinal cords of G93A mice were compared using a Student’s t-test. P-values of < 0.05 were considered statistically significant.

Results
Age-dependent decreases in motor performance and motor neurons in G93A mice
In order to compare the age-dependent profile of motor activity between WT and G93A mice, we investigated the motor performance of these mice. Serial changes in the motor performance of G93A and WT mice from 13 to 19 weeks are shown in Fig. 1. G93A mice did not show typical extension reflex of their hind limbs at 14 weeks and older when held by their tails (Fig. 1A). The ability to remain on the rotarod in G93A mice tended to decrease at 16 weeks and was significantly decreased from 17 weeks and older (Fig. 1B). At week 20, all of the G93A mice showed paralysis and no motor performance was possible. However, no change in the performance was evident in WT mice at any age (Fig. 1B).

We examined serial changes in the expression of SMI32, a neuronal marker protein (28), in the spinal cord of G93A and WT mice at the indicated ages (Fig. 2A). The expression of SMI32 decreased in accordance with age and disease progression in the G93A mice, and significant decrease in the immunoreactivity was observed at 19 weeks. In contrast, the immunoreactivity in WT
mice showed no change at any age.

Typical cresyl violet staining of the spinal cord of G93A mice showed that the majority of the motor neurons in the ventral horn were lost at the end stage (19 weeks) and that vacuolization was apparent in the ventral horn in the lumbar segment (Fig. 2B). In WT mice, by contrast, neurodegeneration was not observed in the spinal cord at any age. Average number of motor neurons per section in the lumbar spinal cord in G93A mice was significantly lower than that in WT mice at 19 weeks (Fig. 2B). In contrast, neurons in the dorsal horn were well maintained in G93A mice as well as in WT at 19 weeks (Fig. 2B). These findings were consistent with those of our previous study (23).

Comparison of temporal protein expression profiles of Iba1 and GFAP in the spinal cord of G93A and WT mice
In order to investigate the roles of microglia in the pathogenesis of ALS, we studied Iba1 protein, a marker of activated microglia, in WT and G93A mice at various ages (Fig. 3A). Western blotting revealed that the level of Iba1 expression in G93A mice increased in an age-dependent manner, significant increases of immunoreactivity being evident at 15, 17, and 19 weeks. On the other hand, in WT mice, the level of Iba1 expression was unchanged at any age. We also investigated the expression level of GFAP, a typical marker protein for astrocytes, in the spinal cord of G93A and WT mice at various ages (Fig. 3B). Although the increase in the expression of GFAP began more slowly than that of Iba1, the levels in G93A mice at 17 and 19 weeks were significantly higher than those in age-matched WT mice.

Characterization of PGES in the spinal cord of G93A mice
To examine the pathological roles of PGESs in the spinal cord of G93A mice, we compared the immunoreactivities of the three major isoforms, mPGES-1, mPGES-2, and cPGES, with those of the respective isoform in WT mice. Western blot analysis showed that the expression level of mPGES-1 in G93A mice was significantly higher than that in G93A mice at 9 weeks and that the higher levels were maintained at 17 and 19 weeks (Fig. 4A). In contrast, the immunoreactivity of mPGES-1 did not change in the spinal cord in WT mice at any age (Fig. 4A). There were no differences in the expression levels of mPGES-2 and cPGES between G93A and WT mice, even in relatively older individuals. Unlike the expression profile of mPGES-1, the protein levels of mPGES-2 and cPGES in the lumbar cord of G93A mice were comparable with those of WT mice at any age (Fig. 4: B, C).

Distribution of up-regulated mPGES-1 in the spinal cord of G93A mice
We sought to identify the distribution of up-regulated mPGES-1 in the lumbar spinal cord of G93A mice. Double-immunofluorescence analysis using antibodies against NeuN, a typical neuronal marker, and mPGES-1 demonstrated mPGES-1 immunoreactivity in NeuN-positive large cells with the typical morphology of motor neurons in the anterior horn of G93A mice at 11 weeks (pre-symptomatic stage) and 15 weeks (early symptomatic stage), whereas in WT mice, mPGES-1 immunoreactivity was hardly evident in these cells (Fig. 5: A, B). Semi-quantitative analysis showed that the intensity of mPGES-1 immunoreactivity in motor neurons in G93A
Characterization of mPGES-1 in ALS

mice was increased at 11 weeks in comparison with age-matched WT mice (Fig. 5C), and significantly higher level of intensity was observed in these neurons at 15 weeks.

We also investigated the localization of mPGES-1 immunoreactivity in Iba1-positive microglia in lumbar spinal cord sections from G93A mice. Co-localization of mPGES-1 and Iba1 immunoreactivities was observed in the anterior horn of G93A mice at 15 weeks, but such co-localization was barely evident at 11 weeks (Fig. 6: A, B). In WT mice, no cells immunoreactive for mPGES-1 and Iba1 were detected in the anterior horn (Fig. 6: A, B). Semi-quantitative analysis of changes in the immunoreactivity of mPGES-1 in activated microglia is shown in Fig. 6D. The relative intensity of mPGES-1 immunoreactivity in G93A mice at 15 weeks was significantly higher than that at 11 weeks (Fig. 6D). Finally, we performed double-immunofluorescence analysis with

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Fig. 2. Sequential changes in SMI32 immunoreactivity and representative cresyl violet-stained sections of the lumbar spinal cord. A: Photographs show representative western blots of SMI32 in the lumbar spinal cord of WT and G93A mice (aged 9 to 19 weeks). Equal amounts of cell lysates (10 μg) were analyzed by western blotting with β-actin as an internal marker. Graphs show the relative densities of bands on the blots estimated quantitatively by Scion imaging software. Quantitative data were normalized and expressed as a percentage of the expression of SMI32 in WT mice aged 9 weeks. Values represent the mean ± S.E.M. (n = 5). *P < 0.05, **P < 0.01. B: Representative cresyl violet-stained sections of the lumbar spinal cord in WT (left) and G93A mice (right). Arrowheads indicate motor neurons. Scale bar indicates 50 μm. DH, dorsal horn; VH, ventral horn. Graphs show numbers of surviving motor neurons in lumbar spinal cord sections in WT and G93A mice at 19 weeks of age. Values represent the mean ± S.E.M. (n = 5). **P < 0.01.
antibodies against mPGES-1 and GFAP, a typical astrocyte marker, in lumbar spinal cord sections from G93A mice at 15 weeks. This revealed that immunoreactivity for mPGES-1 in G93A mice was co-localized in only a few GFAP-positive cells (Fig. 6C).

**Discussion**

A growing body of evidence suggests that mPGES-1 expression is elevated in the brains of patients with Alzheimer’s disease (AD) (29) and multiple sclerosis (MS) (30). Furthermore, it has been reported that mPGES-1 is induced in the cerebral cortex after transient focal ischemia and that up-regulation of mPGES-1 contributes to the exacerbation of stroke injury through production of PGE2 after the ischemia (31). In the present study, we found that in G93A mice, the expression level of mPGES-1 in the spinal cord was elevated prior to the decrease in motor performance and the decrease in the immunoreactivity of SMI32, whereas the level of
Fig. 4. Characterization of PGESs in the lumbar spinal cord of WT and G93A mice. Photographs show representative western blots of mPGES-1 (A), mPGES-2 (B), and cPGES (C) in the lumbar spinal cord of WT and G93A mice (aged 9 to 19 weeks). Equal amounts of cell lysates (mPGES-1, 20 μg; mPGES-2, 10 μg; cPGES, 10 μg; respectively) were analyzed by western blotting, with β-actin as an internal marker. Graphs show the relative densities of bands on the blots estimated quantitatively using Scion imaging software. Quantitative data were normalized and expressed as a percentage of the expression of mPGES-1, mPGES-2, and cPGES in WT mice aged 9 weeks. Values represent the mean ± S.E.M. (n = 5). *p < 0.05, **p < 0.01.
mPGES-1 did not change in the spinal cord of WT mice at any age. Unlike mPGES-1, the levels of cPGES and mPGES-2 did not change at any age in either WT or G93A mice. Previous studies from other laboratories have shown that the levels of PGE$_2$ are increased in the spinal cord from ALS mice (8, 15) and in the cerebrospinal fluid from patients with sporadic ALS (9, 10). A study of mPGES-2–deficient mice demonstrated that mPGES-2 was not essential for PGE$_2$ biosynthesis in peritoneal macrophages (32). Similar to mPGES-2, analysis of cPGES/p23–deleted mice suggests that cPGES is not required for PGE$_2$ synthesis (33). Taken together, these data suggest that increases in the level of mPGES-1 and its product, PGE$_2$, in the spinal cord play a significant role in at least some of the sequential disturbances of motor performance in G93A mice.

**Fig. 5.** Level of mPGES-1 in motor neurons is elevated at the pre-symptomatic and onset stages in G93A mice. Representative confocal images of lumbar spinal cord sections from mice at the pre-symptomatic (11 weeks, panel A) and onset (15 weeks, panel B) stages immunostained with antibodies against mPGES-1 (red) and NeuN (green). The signal for mPGES-1 is co-localized with that of NeuN (yellow). The arrows indicate mPGES-1–positive motor neurons. Scale bar indicates 20 μm.

C: Semi-quantitative analysis of changes in mPGES-1 immunoreactivity in motor neurons. mPGES-1 immunoreactivity was analyzed quantitatively on the basis of fluorescence intensity using Scion imaging software. Quantitative data were normalized and expressed as a percentage of the fluorescence intensity of mPGES-1 in G93A mice aged 15 weeks. Values represent the mean ± S.E.M. (n = 4). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. 

mPGES-1 merge
(A)
WT
G93A
(B)
WT
G93A
(C)
Relative density (%)
0 20 40 60 80 100 120
WT G93A 11
WT G93A 15
It has also been shown that cPLA₂, which plays an important role in supplying arachidonic acid, is up-regulated in the lumbar spinal cord sections before onset of motor coordination in G93A mice and that the cPLA₂ elevation occurred largely in neurons (19). Consistent with these results, we showed a significant increase of mPGES-1 intensity in motor neurons in the ventral horn before onset (11 weeks) of motor impairment in G93A mice, and significantly higher level of the intensity was observed in these neurons at 15 weeks. Taken together, these results suggest that mPGES-1 as well as cPLA₂ in motor neurons may play a role in the pathogenesis to trigger ALS symptoms. This is the first report demonstrating that expression of mPGES-1 is increased in motor neurons during the pre-symptomatic stage and early symptomatic stage in G93A mice.

Activation of glial cells has also been described in ALS mouse models (34, 35). Microglia are resident immune cells of the central nervous system (CNS) and the primary mediators of neuroinflammation (36). Activated microglia are known to release several types of cytokines and inflammatory mediators (37), among which PGE₂...
motor neurons in the spinal cord of ALS model mice. Therefore, we also examined the cellular localization of mPGES-1 at 11 and 15 weeks of age in activated microglia. Unlike motor neuronal mPGES-1, immunoreactivity of mPGES-1 was not detected in Iba1-positive microglia in G93A mice at 11 weeks of age. At 15 weeks of age, mPGES-1 was localized in Iba1-positive microglia as well as motor neurons in G93A mice. In the spinal cords of experimental autoimmune encephalomyelitis (EAE) mice, mPGES-1 expression was substantially up-regulated in macrophages/microglia (30). Taken together, these data suggest that an increase of mPGES-1 in activated microglia also plays a role in the deterioration of motor performance in G93A mice at the early symptomatic stage. It is possible that PGE2 derived from activated microglia enhances motor neuronal death through activation of neuronal EP receptors in G93A mice.

Lipopolysaccharide-evoked synthesis of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) has been shown to trigger induction of mPGES-1 in neurons and microglia in spinal neuroinflammation (39). It has also been shown that cytokines including IL-1β and TNFα were up-regulated in the degenerating spinal cords in G93A mice (40). Our data combined with previous results (40) indicate that the time course of the expression of Iba1 closely follows the profile of the inductions of these pro-inflammatory cytokines. Taken together, it is possible that these pro-inflammatory cytokines contribute to mPGES-1 induction in neurons and microglia in the spinal cord of G93A mice at 15 weeks and older. These results also suggest that activation of microglia and production of cytokines do not contribute to the induction of mPGES-1 in motor neurons observed at 11 weeks. It is unlikely that this temporal change in mPGES-1 expression in motor neurons results from an internal consequence of the mutant SOD1 because the mutant SOD1 is expressed in all types of cells from birth in G93A mice (2).

Although the mechanisms underlying the induction of mPGES-1 in motor neurons and activated microglia still remain to be determined, one possible mechanism is the contribution of excitotoxicity induced by glutamate, one of the major determinants of ALS (41). It has been shown that early growth response gene 1 (EGR-1) is a key transcription factor in regulating the inducible expression of mPGES (42) and that glutamate induces transcription of immediate early genes including EGR-1 by activating N-methyl-D-aspartate receptors in rat hippocampal neurons (43). Therefore, it seems that increased level of extracellular glutamate affects the level of mPGES-1 in motor neurons in the spinal cord of ALS model mice. It has been shown that reducing SOD1 mutant accumulation within neurons by viral-delivered small interfering RNA can slow disease onset (44, 45). In contrast, reducing the mutant SOD1 in astrocytes does not affect onset, but sharply slows subsequent disease progression in ALS mice (46). Unlike microglia, the present results showed that immunoreactivity of mPGES-1 was co-localized only in a few GFAP-positive cells at 15 weeks of age in G93A mice. The present results also showed that the expression of GFAP was increased in G93A mice at 17 weeks and older and that the increase in the level of GFAP contrasted with the decreases in the levels of SMI32 at 17 and 19 weeks of age in G93A mice. Thus, these results also suggest that the increase in the level of GFAP is related to late disease progression. Unlike motor neuronal and microglial mPGES-1, mPGES-1 in astrocytes may not play a pathophysiological role in G93A mice.

It has been shown that the EP2 receptor is expressed in motor neurons in rat organotypic spinal cord cultures (47). Our preliminary studies also showed that the EP2 receptor was expressed in spinal motor neurons of G93A and WT mice at 15 weeks (data not shown). In G93A mice, deletion of the EP2 receptor improved survival and motor strength (48). It is possible that the EP2 receptor-mediated pathway plays a role in facilitating motor neuronal death after onset of motor disturbance. However, further studies are necessary to elucidate mechanisms of the EP2-mediated cell death pathway in the motor neurons.

In conclusion, our present data suggest that mPGES-1, but not other PGESs, mPGES-2 and cPGES, in motor neurons of the spinal cord plays a role in the pathogenesis of ALS and that mPGES-1 may work sequentially in motor neurons and microglia to trigger ALS symptoms. Our findings may provide useful information for elucidating the mechanism of motor neuronal death in ALS and for accelerating the development of new treatments.

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