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A Parkinson's disease gene, DJ-1, regulates anti-inflammatory roles of astrocytes through prostaglandin D_2 synthase expression

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ABSTRACT

Dysfunctional regulation of inflammation may contribute to the progression of neurodegenerative diseases. The results of this study revealed that DJ-1, a Parkinson's disease (PD) gene, regulated expression of prostaglandin D_2 synthase (PTGDS) and production of prostaglandin D_2 (PGD_2), by which DJ-1 enhanced anti-inflammatory function of astrocytes. In injured DJ-1 knockout (KO) brain, expression of tumor necrosis factor-alpha (TNF-α) was more increased, but that of anti-inflammatory heme oxygenase-1 (HO-1) was less increased compared with that in injured wild-type (WT) brain. Similarly, astrocyte-conditioned media (ACM) prepared from DJ-1-KO astrocytes less induced HO-1 expression and less inhibited expression of inflammatory mediators in microglia. With respect to the underlying mechanism, we found that PTGDS that induced expression of HO-1 was lower in DJ-1 KO astrocytes and brains compared with their WT counterparts. In addition, PTGDS levels increased in the injured brain of WT mice, but barely in that of KO mice. We also found that DJ-1 regulated PTGDS expression through Sox9. Thus, Sox9 siRNAs reduced PTGDS expression in WT astrocytes, and Sox9 overexpression rescued PTGDS expression in DJ-1 KO astrocytes. In agreement with these results, ACM from Sox9 siRNA-treated astrocytes and that from Sox9-overexpression astrocytes exerted opposite effects on HO-1 expression and anti-inflammation. These findings suggest that DJ-1 positively regulates anti-inflammatory functions of astrocytes, and that DJ-1 dysfunction contributes to the excessive inflammatory response in PD development.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra (Hirsch, 1988). DJ-1, *PARK7*, is an early onset autosomal-recessive PD-related gene (Bonifati, 2003), which is reported to have diverse functions including regulation of apoptosis, oxidative stress and astrogliosis (Choi et al., 2018; Junn et al., 2005; Mullett and Hinkle, 2009). Some studies have also reported that DJ-1 is a negative regulator of inflammation (Kim et al., 2013b; Kim et al., 2014; Waak et al., 2009).

Brain inflammation that occurs in injured brain protects tissues and repair of injured tissues (Jeong et al., 2013a; Tsarouchas et al., 2018). However, uncontrolled brain inflammation can increase tissue damage and be a risk factor of neurodegenerative diseases such as PD, AD and multiple sclerosis (Breitner, 1996; Chen et al., 2003; Klegeris and McGeer, 2005; Raivich and Banati, 2004; Sheng et al., 1998). Thus, there are two main mechanisms that control brain inflammation through the expression of pro-inflammatory mediators and anti-inflammatory mediators (Min et al., 2006). Astrocytes, the most abundant cells in the brain, produce anti-inflammatory factors of which induce the activation of transcription factor nuclear factor-like 2 (Nrf2) and subsequently induces the expression of antioxidant enzymes such as heme oxygenase-1 (HO-1) in microglia (Min et al., 2006). Thus, alteration or loss of astrocyte anti-inflammatory functions can enhance brain inflammation and contributes to the progression of neurodegenerative diseases.

Abbreviations: PD, Parkinson's disease; PTGDS, Prostaglandin D_2 synthase; PGD_2, Production of prostaglandin D_2; KO, Knockout; TNF-α, Tumor necrosis factor-alpha; HO-1, Heme oxygenase-1; ACM, Astrocyte-conditioned media

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It has been reported that prostaglandin D_2 (PGD_2), which is the most abundant prostaglandin in the CNS (Narumiya et al., 1982), also has anti-inflammatory effects (Kuesap et al., 2008; Satarug et al., 2008). It binds to DP2 receptors and activates the phosphatidylinositol 3-kinase (PI3K)/AKT signal pathway to increase HO-1 expression (Kuesap et al., 2008; Satarug et al., 2008). PGD_2 is produced by two types of PGD synthases (PGDS): hematopoietic-type PGDS (H-PGDS) and prostaglandin D_2 synthase (PTGDS, lipocalin type PGDS, L-PGDS). In the brain, PTGDS is the major enzyme that produces PGD_2 (Urade and Eguchi, 2002; Urade and Hayaishi, 2000), and also protects neurons from oxidative stress and ischemic injury (Fukuhara et al., 2012; Saleem et al., 2009; Taniike et al., 2002). Notably, it has been reported that PGD_2 and PTGDS are linked with neurodegenerative disease and brain injury; PGD_2 levels are increased in AD patients compared with age-matched control (Iwamoto et al., 1989).

In this study, it was found that DJ-1 deficiency weakens the anti-inflammatory function of astrocytes through reduced PTGDS expression and subsequent PGD_2 production. In addition, the transcription factor Sox9 which is stabilized by DJ-1, regulates PTGDS expression.

2. Materials and methods

2.1. Study approval

All experiments were performed in an accordance with approved animal protocols and guidelines established by the Ajou University School of Medicine Ethics Review Committee for animal experiments, and all animal work was approved by the Ethical Committee for Animal Research of Ajou University (2014–0029; AMC119).

2.2. DJ-1 deficient mice

The DJ-1 KO mice (C57BL/6 background, male, 8–10 weeks old) used in this study were a generous gift from Prof. UJ Kang and Prof. X Zhuang (Chicago University, Chicago, IL, USA). DJ-1 KO mice were previously generated by deleting a 9.3-kb region of genomic DNA containing the first five exons and part of the promoter region of the DJ-1 gene (Chen, 2005).

2.3. Cell culture

Primary astrocytes were cultured from the brain of c57/BL6 WT and DJ-1 KO mouse. In briefly, meninges were removed, and brain was mechanically dissociated with gentle pipetting in the media. Dissociated cells in DMEM (Hyclone, Logan, UT, USA) containing 10% FBS (Hyclone) were seeded on 75 cm^2 T-flasks (0.5 hemisphere/flask), and were incubated in 37 °C, 5% CO_2 incubator for 2–3 weeks. Astrocytes were removed with 0.1% trypsin and seeded on culture dishes.

BV2 murine microglia was cultured in DMEM containing 5% FBS as previously described (Min et al., 2006). For activation, cells were treated with 20 ng/ml recombinant murine IFN- γ (PeproTech, Rocky Hill, NJ, USA). PGD2 receptor antagonists (BWA868C for DP1; CAY10471 for DP2) were from Cayman Chemical (Ann Arbor, MI, USA), and PI3K inhibitors (LY294002 and Wortmanin) were from Biomol (Plymouth Meeting, PA, USA).

2.4. Preparation of astrocyte culture conditioned media (ACM)

Astrocytes were seeded on 100 mm dishes (6×10^6 cells/dish). On reaching confluence, media were changed with DMEM without FBS. ACM was collected 5 day after changing the media, and stored at −70 °C until use.

2.5. ELISA

The levels of PGD_2 in ACM were measured using an ELISA kit (Cayman Chemical) according to the manufacturer's instructions.

2.6. DNA constructs

Myc-PTGDS was a gift from Kyoungho Suk (Kyungpook National University School of Medicine, Daegu, Kyungpook, Korea). Myc-Sox9 was modified from pWPXL-Sox9 vector (plasmid # 36979) obtained from Addgene (Watertown, MA, USA). For transfection, cells were transiently transfected with plasmid DNA using the jetPEI transfection reagent (Polyplus-Transfection, San Diego, CA, USA) as described by the manufacturer. Briefly, cells were exposed to plasmid DNA and jetPEI mixture for 4 H. *media* were replaced with fresh DMEM containing 10% FBS. Three days later, transfected cells were used for experiments.

2.7. siRNA tranfection

The expression of PTGDS and Sox9 was knocked down by transiently transfecting astrocytes with specific small interfering RNAs (siRNA; Genolution, Phamaceuticals, Seoul, Korea). The siRNA sequences used in this study were summarized (Supplemental Table 1). For transfections, the medium was replaced with Opti-MEM (Invitrogen, Carisbad, CA, USA) and astrocytes were treated with 10 nM siRNA and RNAi-MAX transfection reagent, according to the manufacturer's instructions (Invitrogen) for 5 days. Knockdown of Sox9 was confirmed by qPCR.

2.8. Western blot analysis

Cells or brain tissues were homogenized in a modified RIPA buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl) containing protease/phosphatase inhibitor cocktails (GenDEPOT, Baker, TX, USA). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. Membranes were incubated with primary antibodies (Supplemental Table 2) overnight at 4 °C. Membranes were washed with Tris-buffered saline containing 0.1% Tween 20 (TBST), incubated with secondary antibodies, and visualized with an enhanced chemiluminescence system (Daeil Lab Inc., Seoul, Korea). Band intensities were measured using Image J.

2.9. Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated from cells and brains using easy-BLUE reagent (iNtRON Biotechnology, Seoul, Korea). cDNA was synthesized using a cDNA synthesis kit (iNtRON) following the manufacturer's guidance. The relevant mRNA levels were measured using a KAP SYBR FAST qPCR kit (Kapa Biosystems, Boston, MA, USA) and a RotoGene thermocycler (Corbett Research, Sydney, Australia). The primer pairs used in this study were summarized (Supplemental Table 3). The cycle threshold (Ct) for the target gene transcript was normalized to the average Ct for β-actin, and the relative quantitation of normalized transcript abundance was determined using the comparative Ct method (ΔΔCt), as described by the manufacturer.

2.10. Stereotaxic injection

Male mice (8–10 weeks old) were anesthetized by intraperitoneal injection of tribromoethanol (250 mg/kg; Sigma, St. Louis, MO, USA) and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Brain injury was produced by unilateral administration of ATP (400 nmole; Sigma) into the striatum (AP, +0.2 mm; ML, −2.5 mm; DV, −3 mm from bregma), according to stereotaxic

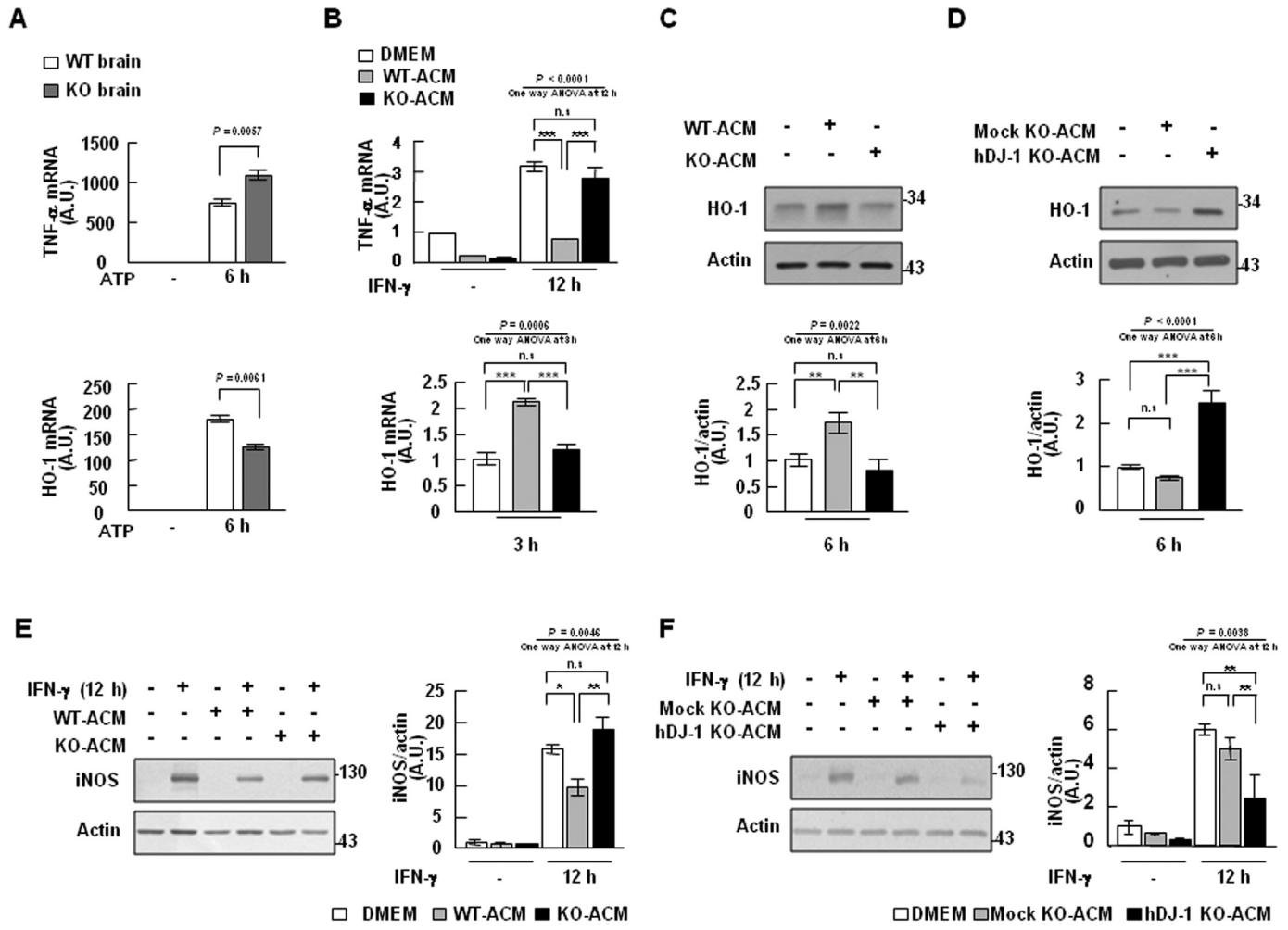

Fig. 1. Anti-inflammatory function of astrocytes is attenuated by DJ-1 deficiency. (A) Brain damage was induced by stereotaxic injection of ATP (400 nmole) into the striatum of WT and DJ-1-KO mice. TNF-α and HO-1 expression was measured at 6 h after ATP injection by qPCR. (B-F) Expression of HO-1, TNF-α, and iNOS was measured in BV2 microglia by qPCR (B) or Western blot and quantified (C-F). For analysis of HO-1 expression, cells were treated with ACM for 6 h. For analysis of TNF-α and iNOS expression, cells were treated with IFN-γ (20 ng/ml) and ACMs for 12 h. WT- and KO ACM (B, C, E) were prepared from WT and DJ-1 KO astrocytes, respectively as described in Material and Methods. Mock- and hDJ-1 ACM (D, F) were prepared from DJ-1 KO astrocytes after transfection. Actin was used as a loading control and the expression was quantified by Image J (B-F). Values are means ± SEMs of three samples. p values were calculated from unpaired two tailed t-test (A) and one way ANOVA followed by a Newman-Keuls post hoc test (B-F),which is contained statistical summary (n.s, nonsignificant; *P < 0.05, **P < 0.005; ***P < 0.001).

coordinates in The Atlas of the Mouse Brain (Paxinos and Franklin, second edition), as previously described (Jeong et al., 2010). All animals were injected using a Hamilton syringe attached to a syringe pump and equipped with a 30-gauge blunt-ended needle to minimize mechanical damage (KD Scientific, New Hope, PA, USA). ATP was dissolved in sterile phosphate-buffered saline (PBS), and 0.8 μl of ATP solution was infused at a rate of 0.2 μl/min. After injection, the needle was held in place for an additional 4 min before removal. The contralateral side was used as a control.

2.11. Tissue preparation

For immunostaining, mice were anesthetized and transcardially perfused first with saline solution containing 0.5% sodium nitrate and heparin (10 U/ml), and then with 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.2). Brains were stored at 4 °C in 4% paraformaldehyde for 2 d, and then in a 30% sucrose solution until they sank. Six separate series of 40-μm coronal brain sections were obtained using a Model CM3050S cryostat (Leica, Wetzlar, Germany) and stored in an anti-freeze stock solution (PB containing 30% glycerol and, 30% ethylene glycol, pH 7.2) at 4 °C before use.

For mRNA and protein preparation, brains were removed and sliced with an Alto mouse brain slicer matrix (SA-2175; Roboz Surgical Instruments, Gaithersburg, MD, USA) and a razor blade. Slices (1 mm thickness) surrounding the needle injection spot were collected and stored at −70 °C until use.

2.12. Immunohistochemistry

Sections or cultures were washed with PBST, treated with 1% BSA, and incubated with combinations of primary antibodies (Supplemental Table 2). Immunoreactive proteins were visualized using Alexa Fluor 488- or Alexa Fluor 555-conjugated secondary antibodies (1:600 dilution; Invitrogen, Carlsbad, CA, USA). Sections were counterstained with 4′, 6-diamidino-2-phenylindol (DAPI; Vector Laboratories) to detect nuclei. Images were captured using a confocal microscope (Carl Zeiss, Oberkochen, Germany). Protein intensities were analyzed using Image J (NIH, Bethesda, MD, USA).

2.13. Differentially expressed genes (DEGs) analysis

For analysis about expression of prostaglandin synthase family

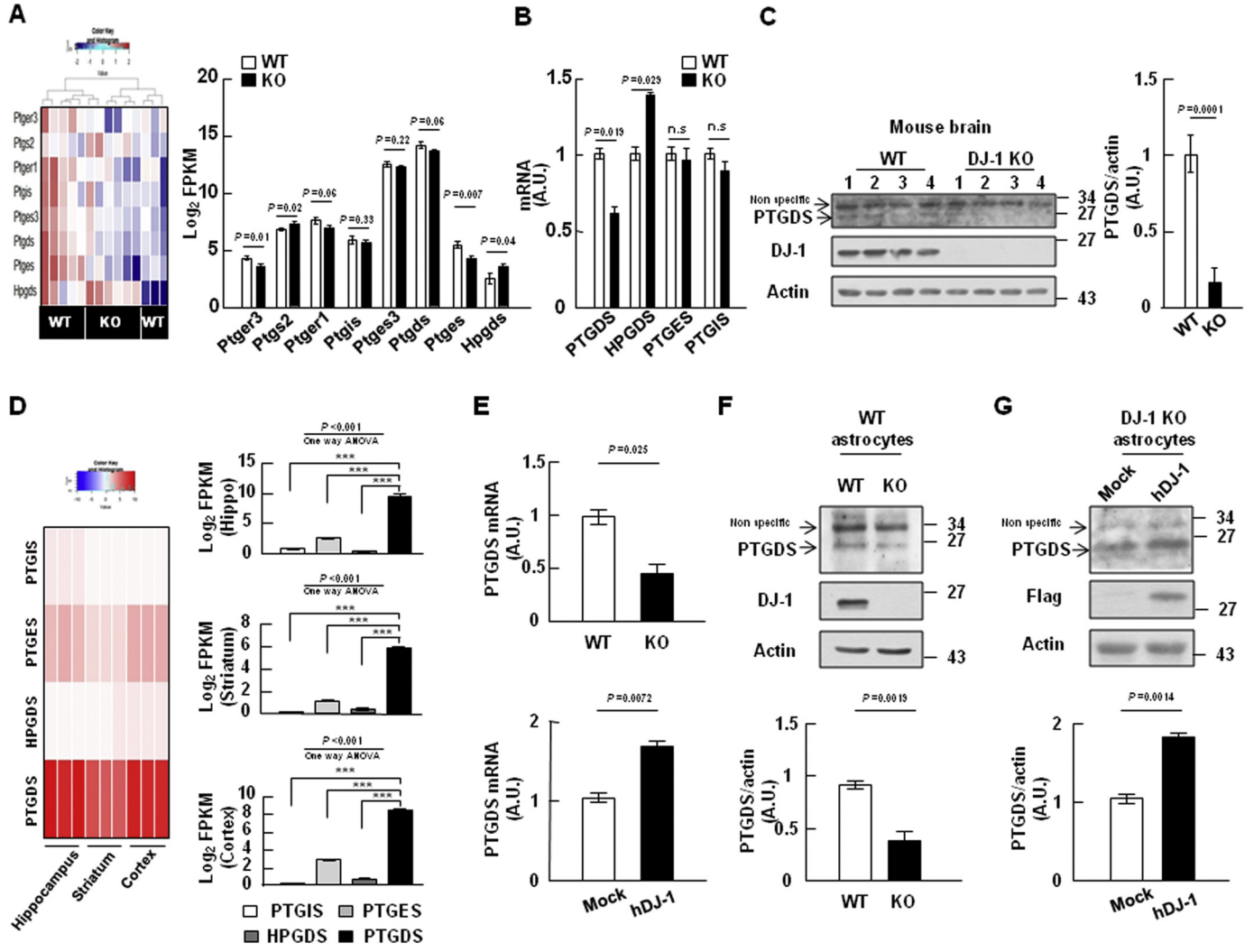

Fig. 2. DJ-1 deficiency attenuates PTGDS expression in mouse brain and astrocytes. (A, D) Heat map (left panel) showed expression of prostaglandin synthases between WT and DJ-1 KO intact rat brain (A) and WT astrocytes (D). Values of $\log_2$ fragments per kilobase million (FPKM) (right panel) showed expression levels of prostaglandin synthases between WT and DJ-1 KO rat brains (A) and WT astrocytes (D). (B, C) mRNA (B) and protein (C) levels of prostaglandin synthases were analyzed in WT and DJ-1 KO mouse brains by qPCR and Western blot. (E-G) Expression of PTGDS in WT and DJ-1 KO astrocytes was analyzed by qPCR (E) and Western blot (F, G). Expression of hDJ-1 but not mock vector (for 3 day) enhanced PTGDS expression in DJ-1 KO astrocytes (E lower panel, G). Actin was used as a loading control, and the expression was quantified by Image J (C, F, G). Values are means ± SEMs of three samples. *p* values were calculated from unpaired two tailed *t*-test (A-C, E-G) and one way ANOVA followed by a Newman-Keuls post hoc test (D), which is contained statistical summary (***$P < 0.001$).

between WT and DJ-1 KO rat brain, NGS dataset was downloaded from GEO (GSE71968) (Hauser et al., 2017). For analysis about expression of prostaglandin synthase in astrocytes on each other region, NGS dataset was downloaded from publishing paper (Clarke et al., 2018). Gene expression was analyzed by R studio.

2.14. Statistical analysis

The statistical significance of differences between two groups was determined using unpaired two-tailed Student's *t*-tests. For comparisons of multiple means, statistical significance was determined by One-way analysis of variance (ANOVA) followed by a Newman-Keuls post hoc test using Graph Pad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Data availability statement.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3. Results

3.1. DJ-1 deficiency attenuates anti-inflammatory function of astrocytes

Inflammation could be enhanced by a decreased in the expression of anti-inflammatory mediators as well as an increase in the expression of pro-inflammatory mediators. In our previous studies, soluble factors from astrocytes prevented excessive inflammation of microglia (Kim et al., 2010; Min et al., 2006; Pyo et al., 2003). Thus, this study examined whether a DJ-1 deficiency altered the anti-inflammatory function of astrocytes. For this, we examined expression levels of tumor necrosis factor-alpha (TNF-α) and heme oxygenase-1 (HO-1) in injured WT and DJ-1 KO brains as markers of pro- and anti-inflammation, respectively (Kim et al., 2010; Min et al., 2006; Pyo et al., 2003). We used ATP, which is a damage-associated molecular patterns (DAMPs), to induce brain damage as reported in previous studies (Choi et al., 2018; Jeong et al., 2010; Jeong et al., 2013b). Interestingly, the expression of TNF-α and HO-1 showed opposing patterns in WT and KO brains at 6 h after ATP injection. TNF-α expression had increased; however, HO-1 expression had decreased in DJ-1 KO brains compared to WT brains

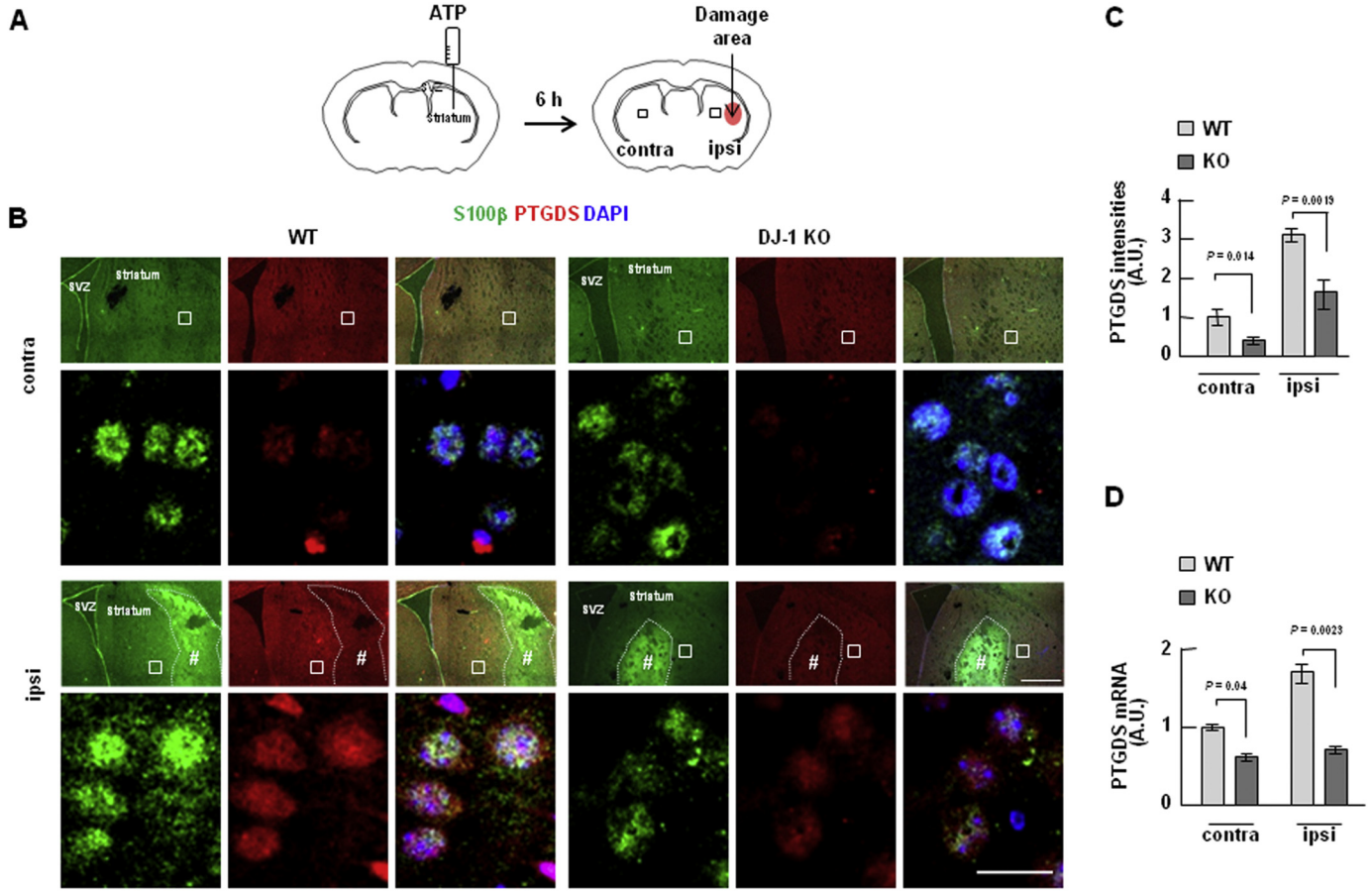

Fig. 3. Attenuated PTGDS expression in astrocytes in intact and injured DJ-1 KO brain. Brain damage was induced by stereotaxic injection of ATP (400 nmole) into the striatum of WT and DJ-1-KO mice. (A) Injury models for assaying PTGDS expression (B) Brain Sections (40 μm) obtained at 6 h after ATP injection and were stained with antibodies specific for S100β, a marker of astrocytes, and PTGDS. (C) Intensities of PTGDS were measured using Image J. (D) mRNA expression of PTGDS was analyzed by qPCR at 6 h after ATP injection. Values are means ± SEMs of three mice. *p* values were calculated from unpaired two tailed t-test (C, D). Scale bar, 500 μm, 20 μm. Contra: contralateral, Ipsi: ipsilaterl, #: damaged region, SVZ: subventricular zone.

(Fig. 1A). Next, we compared the anti-inflammatory effect of astrocyte conditioned media (ACM) prepared from WT (WT-ACM) and DJ-1 KO astrocytes (KO-ACM) by measuring TNF-α and HO-1 expression in BV2 microglia, since HO-1 is a mediator of anti-inflammatory function of astrocytes (Kim et al., 2010; Min et al., 2006; Pyo et al., 2003). Interestingly, KO-ACM had a weak effect on reducing TNF-α expression induced by interferon-gamma (IFN-γ) compared with WT-ACM. Whilst WT-ACM increased HO-1 mRNA and protein expression approximately 2 fold and 1.5 fold within 3, 6 h, but KO-ACM barely increased its expression (Fig. 1B, C). We also found that HO-1 protein expression was rescued by ACM from DJ-1 KO astrocytes transfected with hDJ-1 (hDJ-1 KO-ACM) but not by those transfected with a control vector (Mock KO-ACM) (Fig. 1D). Similar effect was obtained on iNOS expression: compared with WT-ACM, KO-ACM had a weak effect on reducing iNOS expression in IFN-γ-treated microglia (Fig. 1E), and the anti-inflammatory function of KO-ACM was rescued by hDJ-1 KO-ACM (Fig. 1F). These results suggest that attenuation of astrocyte anti-inflammatory function may enhance brain inflammation in DJ-1 deficient mouse brain.

3.2. PTGDS and PGD_2 mediates anti-inflammatory effects of DJ-1

To identify the anti-inflammatory factors of astrocytes that are regulated by DJ-1, we analyzed the RNA sequencing datasets of WT and DJ-1 KO rat brains provided by GEO (GSE71968) (Hauser et al., 2017) and found the possibility that the prostaglandin synthases were a candidate since products of these enzymes induce HO-1 expression and regulate inflammatory responses (Ricciotti and FitzGerald, 2011). Using qPCR analysis, we found reduced mRNA expression of PTGDS in DJ-1 KO brains (Fig. 2B). However, levels of other prostaglandin synthases including prostaglandin E synthase (PTGES) and prostaglandin I_2 synthase (PTGIS) showed little difference in WT and KO brains, and hematopoietic prostaglandin D synthase (HPGDS) levels were rather higher in DJ-1 KO brain (Fig. 2B). In a western blot, PTGDS levels were also lower in DJ-1 KO brains (Fig. 2C). In addition, interestingly, we found that PTGDS was a major prominent prostaglandin synthase of astrocytes in further analysis of RNA sequencing datasets reported by others (Clarke et al., 2018) (Fig. 2D). Based on this information, we examined whether PTGDS could be an anti-inflammatory factor of astrocytes and whether DJ-1 regulated the level of PTGDS. Both western blot and qPCR analyses showed that PTGDS levels in DJ-1 KO astrocytes were < 50% of those in WT astrocytes (Fig. 2E upper panel, Fig. 2F). However, levels of other prostaglandin synthases showed slight change in WT and DJ-1 KO astrocytes (Supplemental Fig. 1A, B). In addition, transfected hDJ-1 increased PTGDS mRNA and protein levels in DJ-1 KO astrocytes (Fig. 2E lower panel, G).

Next, we analyzed PTGDS expression in intact and injured brains. Brain sections were obtained at 6 h after ATP injection, and stained with antibodies specific for PTGDS and S100β, a marker of astrocytes. PTGDS expression was found in S100β-positive astrocytes in intact brain, and its level increased in response to injury (Fig. 3A-C). However, PTGDS levels in DJ-1 KO brains were lower than in WT brains in both intact (Contra) and injury states (Ipsi) (Fig. 3A-C). As for staining, PTGDS mRNA levels in DJ-1 KO brains were lower in intact states than

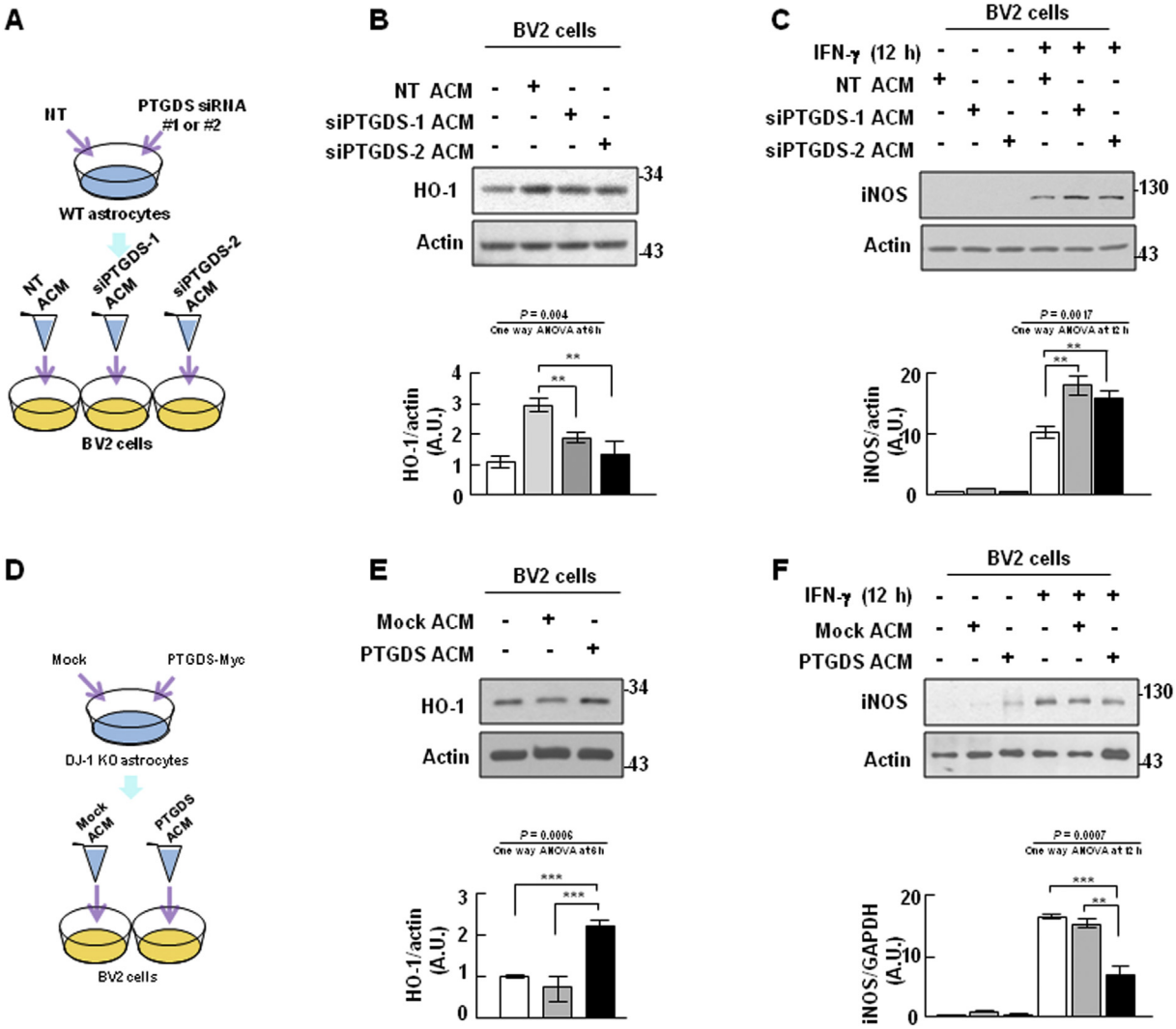

Fig. 4. PTGDS regulates anti-inflammatory function of astrocytes. (A, C) Experimental designs. Effect of ACM on HO-1 expression (B, E) and IFN-γ-induced iNOS expression (C, F) was measured in BV2 microglia with western blot. (A, B) ACMs were obtained from WT astrocytes transfected with siRNAs specific of PTGDS (siPTGDS-1 and siPTGDS-2, 10 nM each) or non-targeted siRNA (NT) for 5 day. (C, D) ACMs were obtained from DJ-1 KO astrocytes transfected with PTGDS and mock vector. Actin and GAPDH were used as a loading control. The expression was quantified by Image J. Values are means ± SEMs of three samples. *p* values were calculated from one way ANOVA followed by a Newman-Keuls post hoc test (B, C, E, F), which is contained statistical summary (**$P < 0.005$; ***$P < 0.001$).

in WT brains (Fig. 3D). In addition, PTGDS mRNA levels had increased in ATP-injected WT brains but not KO brains (Fig. 3D). However, mRNA levels of PTGES, PTGIS, and HPGDS were similar in the WT and DJ-1 KO brains in both intact and injury states (Supplemental Fig. 2).

We further confirmed PTGDS as an active anti-inflammatory factor of astrocytes with knock down of PTGDS in WT astrocytes (Fig. 4A, Supplemental Fig. 3A, B) and/or overexpression of PTGDS in DJ-1 KO astrocytes (Fig. 4D, Supplemental Fig. 3C). siRNAs specific for PTGDS effectively decreased the expression of PTGDS in astrocytes (Supplemental Fig. 3A, B). ACMs (siPTGDS-1/2 ACM) prepared from PTGDS siRNAs treated astrocytes less induced HO-1 expression (Fig. 4B), and less inhibited IFN-γ-induced iNOS expression in microglia than the ACM from non-targeted (NT) siRNA-treated astrocytes (Fig. 4C). In addition, ACM (PTGDS ACM) from PTGDS-overexpressing DJ-1 KO astrocytes significantly induced HO-1 expression and inhibited iNOS expression than empty-vector expressing (Mock) astrocytes (Fig. 4E, F).

It has been reported that PGD_2, a product of PTGDS (Eguchi et al., 1999; Eichele et al., 2008), induces HO-1 expression in several types of cells (Kuesap et al., 2008; Satarug et al., 2008). Therefore, we measured the levels of PGD_2 in WT- and DJ-1 KO ACM using ELISA: PGD_2 levels in DJ-1 KO ACM were about 40–50% of that in WT ACM (Fig. 5A). In addition, ACM-induced HO-1 expression in microglia was inhibited by DP2 PGD_2 receptor antagonists, CAY10471, but not by a DP1 antagonist, BW A868C (Fig. 5B). Since PGD_2 induces HO-1 expression through AKT activation (Jeong et al., 2018), we further examined the levels of AKT activation in WT ACM- and KO ACM-treated microglia. As expected, DJ-1 KO ACM less increased phosphor-AKT (pAKT) levels than WT ACM (Fig. 5C). In addition, WT ACM-induced HO-1 induction was reduced by wortmannin and LY294002, inhibitors of PI3K, an upstream kinase of AKT (Fig. 5D). Taken together, these results suggest that DJ-1 deficiency reduced PGD_2 production and PTGDS from astrocytes, resulting in reduced microglial HO-1 expression and subsequently attenuated anti-inflammatory function.

3.3. *Sox9 regulates PTGDS expression in astrocytes*

Next, we investigated how DJ-1 regulates PTGDS expression in astrocytes. It has been reported that Sox9 regulates mRNA levels of PTGDS (Wilhelm et al., 2007). Furthermore, we recently reported that DJ-1 is important for stabilization of Sox9 protein through inhibition of Sox9 ubiquitination (Choi et al., 2018). Based on the information, we analyzed whether the defect of Sox9 in DJ-1 KO astrocytes was related

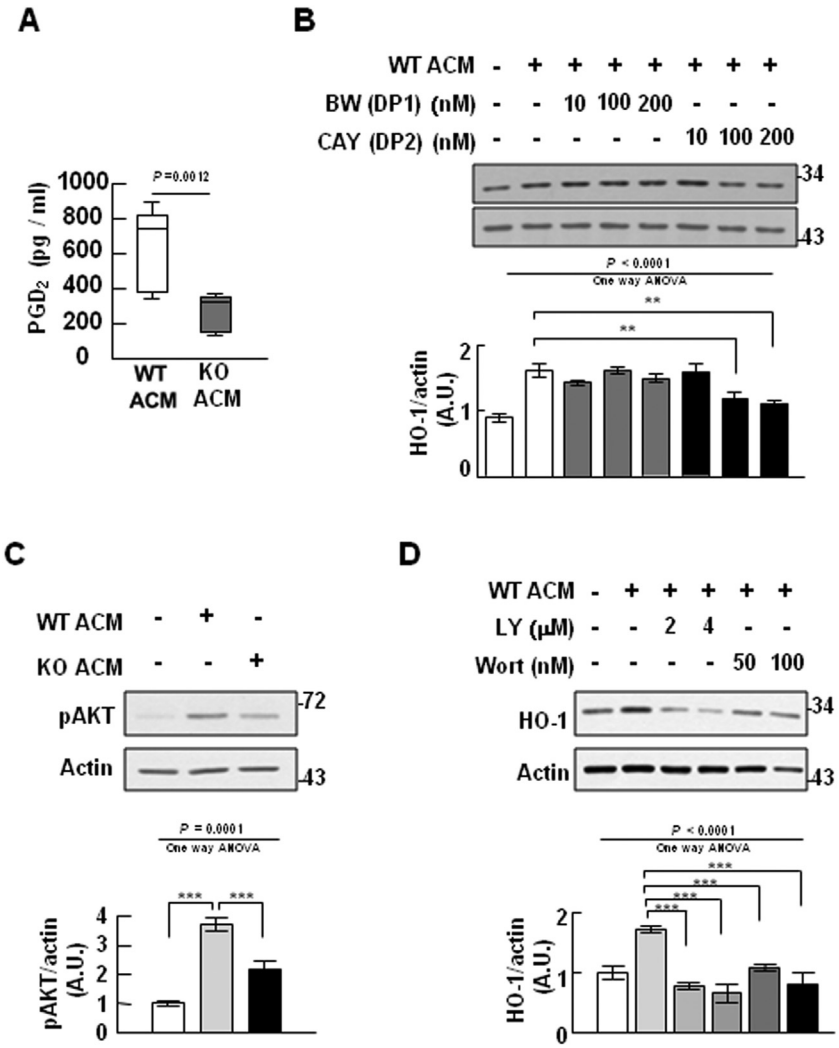

Fig. 5. DJ-1 deficiency attenuated astrocyte PGD_2 production. (A) The amount of PGD_2 in WT and DJ-1 KO ACM was measured with ELISA. (B, D) BV2 microglia was incubated with ACM for 6 h in the absence or presence of antagonists of PGD_2 receptors (CAY1047 for DP2; BWA868C for DP1, 10, 100 and 200 nM each) (B) or PI3K inhibitors, wortmannin and LY294002, as indicated (D). **(C)** pAKT levels were measured in microglia at 30 min after ACM treatment. HO-1(B, D) and pAKT (C) levels were analyzed by Western blot. Actin was used as a loading control. The expression was quantified by Image J. Values are means ± SEMs of three samples. *p* values were calculated from unpaired two tailed t-test (A) and one way ANOVA followed by a Newman-Keuls post hoc test (B-D), which is contained statistical summary (**P < 0.005; ***P < 0.001).

to the reduction in PTGDS expression using siRNA specific for Sox9. Interestingly, Sox9 siRNA (siSox9 1/2) reduced mRNA levels of PTGDS in WT astrocytes (Fig. 6B). Accordingly, ACM (siSox9–1/2 ACM) obtained from siSox9 treated astrocytes less reduced iNOS expression in IFN-γ-treated microglia compared with ACM from siNT-treated astrocytes (Fig. 6C), and less induced HO-1 expression (Fig. 6D). In addition, altered PTGDS expression was rescued by Sox9 overexpression in DJ-1 KO astrocytes (Fig. 6F). Accordingly, ACM from Sox9-overexpressing astrocytes significantly reduced IFN-γ-induced iNOS expression, and more strongly increased HO-1 expression in microglia (Fig. 6G, H). Taken together, these results indicate that DJ-1 deficiency reduced Sox9 levels and subsequently PTGDS expression in astrocytes, by which DJ-1 deficiency reduced anti-inflammatory function of astrocytes. (See Fig. 7.)

4. Discussion

The results of this study show that a DJ-1 deficiency in astrocytes reduced PTGDS expression and subsequent PGD_2 secretion, by which astrocytic anti-inflammatory functions were attenuated. In addition, PTGDS expression was closely related with Sox9 levels in astrocytes. Thus, reduced Sox9 levels in DJ-1 KO astrocytes resulted in reduced PTGDS expression.

Inflammation accompanied with injury may aggravate the injury (Breitner, 1996; Chen et al., 2003; Klegeris and McGeer, 2005; Raivich and Banati, 2004; Sheng et al., 1998). Therefore, brain inflammation should be tightly regulated since neurons are rarely regenerated once damaged. There are diverse mechanisms that regulate brain inflammation: turning on expression of negative regulators of inflammation and turning off signaling pathways for activation of inflammation in microglia and astrocytes, etc., (Yang et al., 2007). It has been reported that DJ-1 regulates inflammation in several ways: DJ-1 negatively regulates STAT1 activation through enhancing interaction between STAT1 and its phosphatase, SHP1 (Kim et al., 2013b), and/or enhancing expression of SOCS1, a negative regulator of STAT1, through mir-155 (Kim et al., 2014). Since astrocytes induce anti-inflammatory molecules in microglia (Min et al., 2006), and DJ-1 is expressed in astrocytes (Waak et al., 2009), we hypothesized DJ-1 may regulate inflammation through anti-inflammatory roles of astrocytes. As expected, DJ-1 KO ACM induced HO-1 expression in microglia less strongly than WT ACM (Fig. 1B, C), subsequently exerted less inhibitory effect on IFN-γ-induced iNOS and TNF-α expression (Fig. 1B, E). In addition, defective anti-inflammatory functions of KO ACM were rescued by DJ-1 overexpression (Fig. 1D, F). Therefore, it is evident that DJ-1 regulates brain inflammation via two mechanisms: direct regulation of pro-inflammatory mediator expression in microglia, and indirect regulation of anti-inflammatory functions of astrocytes.

With regard to the anti-inflammatory factors produced by astrocytes, several molecules have been suggested, transforming growth factor (TGF)-β (da Cunha and Vitkovic, 1992; Vincent et al., 1997), dehydroepiandrosterone (DHEA), a neurosteroid (Zwain and Yen, 1999), and prostaglandins including PGE_2 (Molina-Holgado et al.,

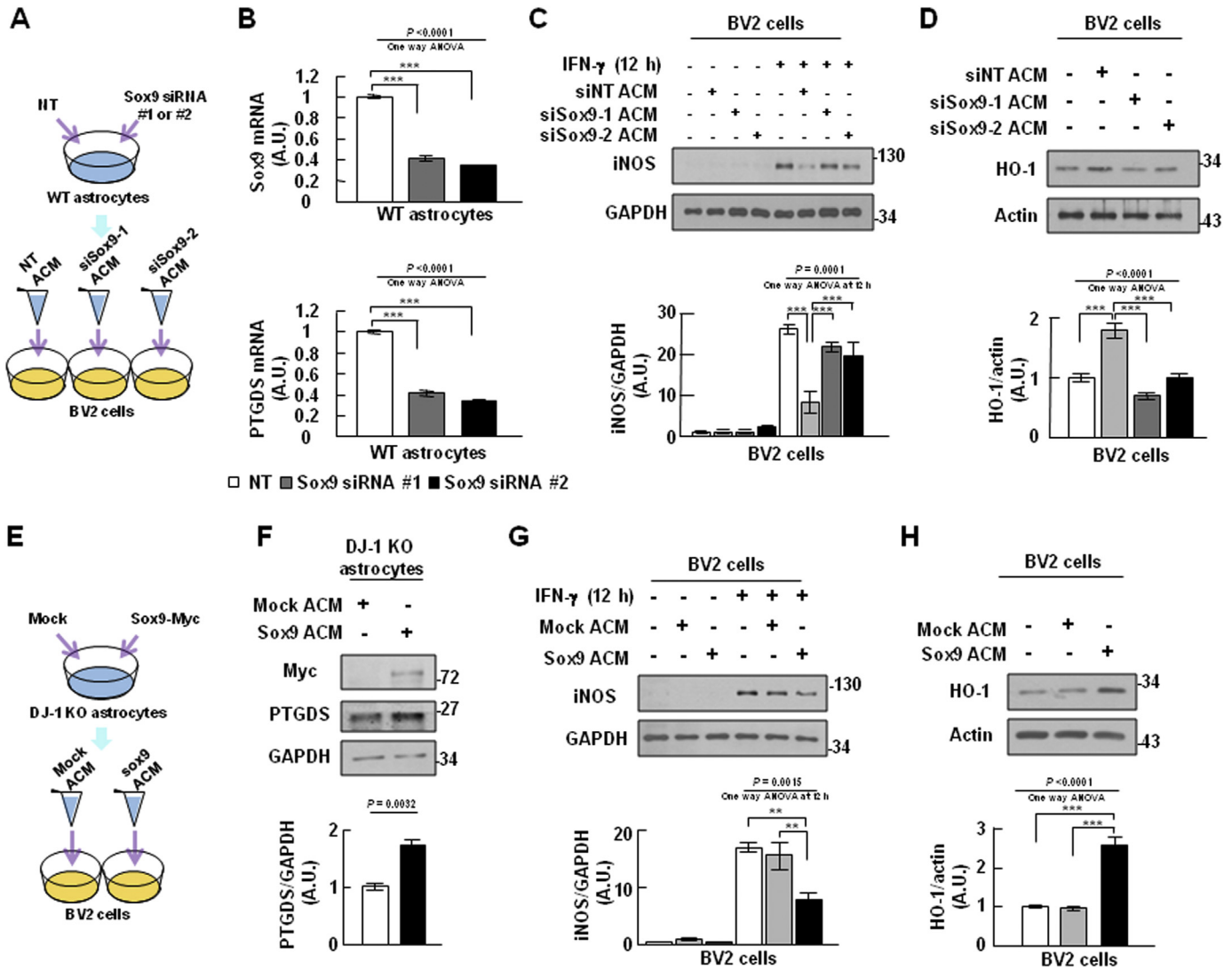

Fig. 6. Sox9 regulates PTGDS expression in astrocytes. (A, E) Experimental designs. (B) WT Astrocytes were treated with Sox9 specific siRNAs (siSox9–1 and siSox9–2, 10 nM each) or NT-siRNA for 5 day. mRNA levels of PTGDS and Sox9 were measured with qPCR, and ACMs were obtained. (F) DJ-1 KO astrocytes were transfected with Myc-Sox9 and mock vector for 3 day. PTGDS and Myc levels were measured with western blot, and ACMs were obtained. Effect of ACMs obtained from WT astrocytes after transfection with siSox9 and si-NT (C, D), or DJ-1 KO astrocytes transfected with Myc-Sox9 and mock vector (G, H) on IFN-γ-induced iNOS expression (C, G) and HO-1 expression (D, H) and were analyzed. Actin and GAPDH were used as a loading control. The expression was quantified by Image J. *p* values were calculated from unpaired two tailed t-test (F) and one way ANOVA followed by a Newman-Keuls post hoc test (B-D, G, H), which is contained statistical summary (, **P < 0.005; ***P < 0.001).

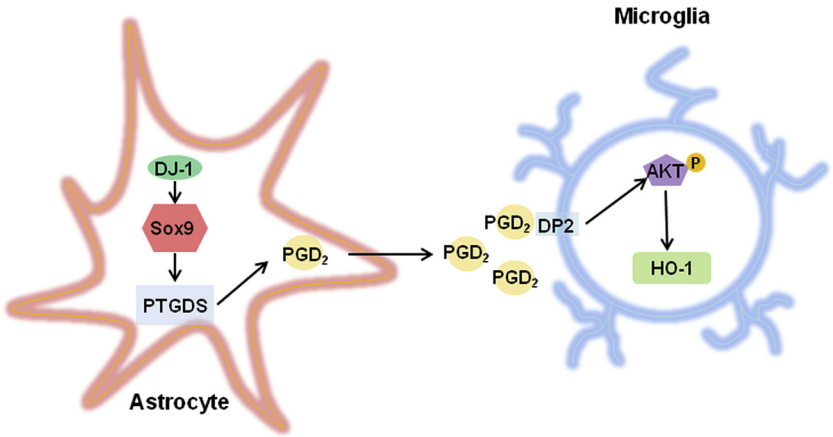

Fig. 7. Models of DJ-1 mediated anti-inflammatory function of astrocytes. In astrocytes, DJ-1 stabilizes Sox9, by which regulates expression of PTGDS, an enzyme that catalyzes PGD_2 production. PGD_2 from astrocytes activates DP2 receptors in microglia, by which HO-1 is expressed through AKT activation.

2000; Taniura et al., 2002). These molecules exert anti-inflammatory effects regulating signaling pathways such as JAK-STAT, MAP kinase and NF-kB. (Altman, 2008; Qian et al., 2008). Our previous study reported that soluble factors produced by astrocytes in intact and injury states was heat-labile and smaller than 3 kDa, and induced HO-1 in microglia (Kim et al., 2010; Min et al., 2006). The results of this study showed that PGD_2 could be an active molecule in ACM because it is released from astrocytes, and induced HO-1 expression in microglia (Fig. 5A-D). In addition, levels of PGD_2 were reduced in DJ-1 KO ACM (Fig. 5A) and were due to reduced levels of PTGDS (Fig. 2). Although PGD_2 is also produced by another prostaglandin D_2 synthase, HPGDS, HPGDS levels were rather slightly increased in (Fig. 2, Supplemental Fig. 1) and its induction rate was not changed in intact or injured DJ-1 KO brain compared with WT (Supplemental Fig. 2). In addition, PTGDS levels increased in response to injury in WT brain but barely in the DJ-1 KO brain (Fig. 3), which may reduce anti-inflammatory function of DJ-1 KO astrocytes and subsequently increase inflammation in injured DJ-1 KO brain.

Next arising question was how DJ-1 regulates PTGDS expression in astrocytes. Our previous study revealed that DJ-1 stabilized Sox9 at protein level through inhibition of Sox9 ubiquitination (Choi et al., 2018), but not directly binding effect (Supplemental Fig. 4). Furthermore, it has been reported that in sertoli cells PTGDS expression is regulated by Sox9 (Wilhelm et al., 2007). In astrocytes, Sox9 regulates PTGDS expression: PTGDS levels were reduced by Sox9 siRNA in WT astrocytes (Fig. 6B), and enhanced by Sox9 overexpression in DJ-1 KO astrocytes (Fig. 6F). Accordingly, Sox9 siRNA and overexpression showed opposite effects on HO-1 expression and anti-inflammatory functions of ACM (Fig. 6C, D vs. Fig. 6G, H). These results suggest that DJ-1 deficiency decrease PTGDS expression and PGD_2 production due to Sox9 protein destabilization. We and others have reported diverse functions Sox9 in the brain including regulation of gliogenesis during brain development (Cheng et al., 2009; Kang et al., 2012), regulation of astrogliosis in injured brain (Kordes and Hagel, 2006; McKillop et al., 2013), and expression of growth factors including BDNF and GDNF (Choi et al., 2018). The present findings revealed another function of Sox9, regulation of brain inflammation through the expression of PTGDS which catalyzes PGD_2 production and subsequently increases HO-1 expression. Taken together, Sox9 could be a target to regulate various functions of astrocytes and produce positive effects in the injured brain.

5. Conclusions

Dysfunction of glia including astrocytes and microglia may play critical roles in the onset and progression of neurodegenerative diseases including PD (Joe et al., 2018). Mutation in PD genes such as PINK1, DJ-1, alpha-synuclein, and LRRK2 alter the microglial inflammatory response and surveillance function (Choi et al., 2015; Kim et al., 2012; Kim et al., 2013a; Kim et al., 2013b; Kim et al., 2014; Kim et al., 2013c). Mutation in PD genes also affects astrocyte functions such as metabolism, mitochondrial function, gliogenesis, astrogliosis, phagocytosis. (Choi et al., 2018; Choi et al., 2016; Choi et al., 2013; Kim et al., 2016). The findings of this study add one more evidences that supports mutation of a PD gene alters glial function, anti-inflammatory function of astrocytes.

Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2019.04.003.

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