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# DJ-1 regulates the expression of renal (pro)renin receptor via reactive oxygen species-mediated epigenetic modification

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ABSTRACT

*Background:* DJ-1 protein plays multifunctional roles including transcriptional regulation and scavenging oxidative stress; thus, it may be associated with the development of renal disorders. We investigated whether DJ-1 protein regulates the expression of (pro)renin receptor (PRR), a newly identified member of renin–angiotensin system.
*Methods:* The levels of mRNA and protein were determined by real-time PCR and western blot, respectively. $H_2O_2$ production was tested by using fluorescence probe. Histone modification was determined by chromatin immunoprecipitation.
*Results:* The expression of PRR was significantly higher in the kidney from DJ-1 knockout mice (DJ-$1^{-/-}$) compared with wild-type mice (DJ-$1^{+/+}$). Histone deacetylase 1 recruitment at the PRR promoter was lower, and histone H3 acetylation and RNA polymerase II recruitment were higher in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$. Knockdown or inhibition of histone deacetylase 1 restored PRR expression in mesangial cells from DJ-$1^{+/+}$. $H_2O_2$ production was greater in DJ-$1^{-/-}$ cells compared with DJ-$1^{+/+}$ cells. These changes in PRR expression and epigenetic modification in DJ-$1^{-/-}$ cells were induced by $H_2O_2$ treatment and reversed completely by addition of an antioxidant reagent. Prorenin-stimulated ERK1/2 phosphorylation was greater in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$ cells and this was inhibited by a PRR-inhibitory peptide, and by AT1 and AT2 receptor inhibitors. The expression of renal fibrotic genes was higher in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$ cells and decreased in PRR-knockdown DJ-$1^{-/-}$ cells.
*Conclusions:* We conclude that DJ-1 protein regulates the expression of renal PRR through $H_2O_2$-mediated epigenetic modification.
*General significance:* We suggest that renal DJ-1 protein may be an important molecule in the acceleration of renal pathogenesis through PRR regulation.

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## 1. Introduction

The renin–angiotensin system (RAS) plays a critical role in the initiation and progression of renal disease [1]. The (pro)renin receptor (PRR), a newly identified member of the RAS, is a single transmembrane receptor for renin and its precursor prorenin [2]. Binding of renin and prorenin to the PRR leads to two distinct reactions: it facilitates the catalytic activity of renin or prorenin in converting angiotensinogen to angiotensin (Ang) I and it directly transmits its signals into the intracellular space [3,4]. Both pathways consequently initiate the activation of extracellular signal-regulated kinase (ERK) 1/2, in Ang II type (AT) 1 receptor-dependent and/or -independent manner [5]. PRR-activated ERK1/2 also participates in the production of the fibrotic factors in renal mesangial cells stimulated with prorenin [6–8]. Moreover, the overexpression of PRR in animals showed renal pathogenesis, such as glomerulosclerosis, fibrosis, or proteinuria [9,10]. Although previous reports have considered a pivotal role of the PRR in renal pathogenesis, a regulatory mechanism of the PRR by reactive oxygen species (ROS) or antioxidant proteins, e.g. DJ-1 protein, has not been elucidated.

DJ-1 was described originally as an oncogene that transforms NIH3T3 cells in cooperation with H-ras [11] and has also been found to be a causative gene for familial Parkinson's disease [12]. DJ-1 is a multifunctional antioxidant protein that scavenges ROS [13]. It is commonly accepted that DJ-1 deficiency results in the elevation of ROS in a variety of cells including renal, vascular, and immune cells [14–16]. Previous studies have reported that ROS in the absence of DJ-1 contributes to diverse pathophysiological events including the overexpression of genes [14,17–19]. In addition, DJ-1 regulates transcriptional activities of several genes by directly interacting with histone deacetylase

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(HDAC) [20,21]. Thus, these data suggest that DJ-1 may be involved in the acceleration of renal diseases resulting from the ROS-mediated transcriptional regulation.

Epigenetic modifications such as acetylation, methylation, ubiquitination, and phosphorylation of histone protein are associated with the development of diverse diseases [22]. Acetylation of histone proteins is regulated mainly by the balance between HDAC and histone acetyltransferase (HAT) [23]. The regulation of HDAC contributes to nuclear and cellular processes such as gene expression, development, cell cycle, and migration [24]. Therefore, clarifying the epigenetic roles of DJ-1 is important if we are to understand the acceleration of pathogenesis in the kidney.

In the present study, we tested the hypothesis that DJ-1 deletion is linked to the upregulation of the renal PRR, which consequently results in renal pathogenesis. We monitored the epigenetic regulation of the PRR in mesangial cells from DJ-1 knockout (DJ-$1^{-/-}$) and their wild-type mice (DJ-$1^{+/+}$) and identified the possible implications of DJ-1 protein in renal diseases.

## 2. Materials and methods

### 2.1. Materials

Valproic acid sodium salt (VPA), mouse handle-region peptide (mH-RP), PD123319, losartan, N-acetyl-cysteine (NAC), and $H_2O_2$ were purchased from Sigma (St. Louis, MO, USA). Recombinant mouse prorenin was obtained from R&D Systems (Minneapolis, MN, USA). HDAC 1 antibody was purchased from Abcam (Cambridge, UK). Anti-acetyl-histone H3 and -RNA polymerase II (RNAPII) antibodies were obtained from Millipore (Bedford, MA, USA). Other antibodies including PRR were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). DMEM F12 was obtained from Gibco (Carlsbad, CA, USA).

### 2.2. Animals and sample preparation

All animal experiments were performed in accordance with the institutional guidelines of Konkuk University, Seoul, Korea. This study conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and was approved by the Animal Subjects Committee of Konkuk University School of Medicine. DJ-$1^{-/-}$ (C57BL/6. B6.cg-Park7tm1shn/J, n = 42) and DJ-$1^{+/+}$ (C57BL/6, n = 38) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Male mice at 6–8 weeks old were used in all experiments. Mice were anesthetized by IP injection of Zoletil (40 mg/kg body weight; Virbac Laboratories, Carros, France) and Rompun (10 mg/kg body weight; Bayer Korea, Korea). The collected tissue samples were kept at −70 °C. Genotyping was performed in genomic DNA from the tails of the mice (Supplementary Fig. S1A). DJ-1 deficiency was confirmed by western blot analysis (Supplementary Fig. S1B).

### 2.3. Isolation of renal mesangial cells

The renal cortex was dissected from the kidneys of 6–8-week-old mice and was minced in DMEM F12 containing 20% FBS and treated with 0.1% collagenase (Wako, Japan) at 37 °C for 90 min. The glomeruli were isolated from the kidneys under sterile conditions by gradual sieving (32, 53, 70, and 100 μm) as described previously [25]. The glomeruli were resuspended in medium containing 20% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured at 37 °C under a humidified 95%/5% (v/v) mixture of air and $CO_2$. All cells were used from passage 2 to 8. Immunocytochemistry was performed with anti-α-smooth muscle actin (α-SMA), -Wilms tumor (WT) 1, or -platelet endothelial cell adhesion molecule (PECAM) to identify the mesangial cells (Supplementary Fig. S1C). The purity of α-SMA-positive mesangial cells isolated was more than 95%.

### 2.4. Immunohistochemistry

Tissues from mice were fixed in 4% paraformaldehyde solution in PBS for 24 h, washed with PBS for 24 h, and embedded in paraffin blocks. The blocks were cut to 5-μm thickness and each specimen was mounted on a glass slide. The specimen was blocked for 5 min at room temperature (RT) with Peroxidase Blocking Solution containing $H_2O_2$ and 15 mM $NaN_3$ (Dako REAL™, Copenhagen, Denmark) and incubated with PRR antibody (1:100) and then labeled polymer-HRP (Dako REAL™) for 30 min at RT. Negative controls were incubated with matching IgG as a primary antibody (Supplementary Fig. S2). The specimen was incubated with DAB and counterstained with hematoxylin. Immunostained specimens were observed using a microscope system. For image analysis, the glomerular DAB intensity was measured using MetaMorph imaging software (version 7.1; Molecular Devices, Downingtown, PA, USA).

### 2.5. Western blot analysis

The frozen tissues or cells were homogenized in radioimmunoprecipitation assay buffer containing protease inhibitors. Lysates were centrifuged (14,000 rpm, 15 min, 4 °C) and supernatants were collected as protein samples. Protein concentration was measured using Bio-Rad DC protein assay reagents (Bio-Rad Laboratories, Hercules, CA, USA). The homogenates were denatured with SDS sample buffer containing 40 mM Tris–HCl (pH 6.8), 8 mM EGTA, 4% 2-mercaptoethanol, 40% glycerol, 0.01% bromophenol blue, and 4% SDS, and then boiled for 5 min. Equal amounts of samples were subjected to 8–10% SDS-PAGE and then transferred electrophoretically to a PVDF membrane (Millipore). The membrane was blocked for 1 h at RT with PBS containing 5% bovine serum albumin and incubated with primary antibodies of anti-PRR (1:500), anti-HDAC1 (1:1000), anti-P-ERK (1:1000), anti-T-ERK (1:2000), or anti-β-actin (1:3000) overnight at 4 °C. Immune complexes were incubated with HRP-conjugated antibody (1:1000) for 1 h at RT. After application of the secondary antibody, the blots were incubated in ECL reagents (Amersham Pharmacia, Piscataway, NJ, USA) and exposed to photographic film. Band intensity was quantified by optical densitometry using ImageJ software (NIH, Bethesda, MD, USA).

### 2.6. Quantitative real-time PCR

RNA of renal tissues and cells was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Total RNA (1 μg) was reverse transcribed into cDNA using the SuperScript™ III First-Strand Synthesis System (Invitrogen). Real-time PCR was performed using the iQ™ SYBR Green Supermix and iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories). All samples were amplified in triplicate in a 96-well plate under the following conditions: 3 min at 95 °C and 40 cycles at 95 °C for 10 s followed by 30 s at 60 °C. The relative mRNA expression level was determined by calculating the value of the Δcycle threshold (ΔCt) by normalizing the average Ct value by its endogenous control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then calculating $2^{-\Delta\Delta Ct}$ [26]. The values were represented as fold-changes based on the control group relative to the experiment groups. The following primer sets were used in the real-time PCR: type 1 collagen (Col1a; NM_007742) forward, 5′-ACCAGCAGACTGGCAACCTC-3′, reverse, 5′-CCCAAGTTCCGG TGTGACTC-3′; fibronectin 1 (Fn1; NM_010233) forward, 5′-ATCTTTCC AGCCCCACCCTA-3′, reverse, 5′-CATGGCTCCAGAGCAAAAGG-3′; GAPDH (NM_008084) forward, 5′-TGATGGGTGTGAACCACGAG-3′, reverse, 5′-GGGCCATCCACAGTCTTCTG-3′; plasminogen activator inhibitor-1 (PAI-1; M33960) forward, 5′-TTGGCAACCCACGTTAAAGG-3′, reverse, 5′-CACAGCCCCTTTTCCCTCTC-3′; PRR (NM_027439) forward, 5′-GCGT TATGGGGAAGACTCTG-3′, reverse, 5′-CACAAGGGATGTGTCGAATG-3′;

and transforming growth factor-β1 (TGF-β1; NM_011577) forward, 5′-AGAGCTGCGCTTGCAGAGAT-3′, reverse, 5′-GTCAGCAGCCGGTTACCAAG-3′.

### 2.7. ChIP assay

Chromatin immunoprecipitation (ChIP) analysis was performed using an EZ ChIP (Millipore) according to the manufacturer's protocols with some modifications. Tissues or cells were fixed with 1% formaldehyde, homogenized, suspended in SDS lysis solution, and sonicated to shear genomic chromatin into the appropriate DNA fragments. The lysate was incubated with protein G agarose beads for 2 h and, after removal of the beads, the lysate was incubated with anti-acetyl-histone H3 (1 μg), -RNAPII (1 μg), or -HDAC1 (1 μg) antibodies at 4 °C overnight. Rabbit IgG was also incubated as a negative control. Protein G beads were added and the mixture was incubated for 1 h. The beads were washed sequentially with a low-salt solution, high-salt solution, LiCl solution, and Tris–EDTA solution. The antibody–chromatin complexes were eluted from the beads with an elution buffer (1% SDS and 0.1 M $NaHCO_3$), 0.2 M NaCl was added, and the complexes were incubated at 65 °C for 5 h to dissociate DNA from protein. The proteins were eliminated by digestion with proteinase K at 45 °C for 2 h, and the DNA was purified with a spin column. The PRR promoter regions were amplified by quantitative real-time PCR, as described above. All samples were amplified in triplicate in a 96-well plate under the following conditions: 3 min at 95 °C and 60 cycles at 95 °C for 10 s followed by 30 s at 60 °C. Quantitative real-time PCR values were normalized to input. The primer sets used in real-time PCR were PRR (NC_000086) forward, 5′-CCGCCCTGTTCTTAAAACTC-3′ and reverse, 5′-CTTTAGCCGCTCCAAGACAA-3′.

### 2.8. siRNA transfection

Transfection was performed by using Welfect-Q (Welgene, Korea) according to the modified manufacturer's protocols. HDAC1 siRNA or non-silencing scrambled siRNA (100 nM) was transfected into cells and studied after 48 h. Transfection efficiency was confirmed by HDAC1 level using western blot.

### 2.9. Measurement of $H_2O_2$ levels

Intracellular $H_2O_2$ levels were measured using the cell-permeable fluorescence probe 6-carboxy-2′,7′-dichlorofluorescein diacetate (DCF-DA; Invitrogen). Mesangial cells were plated into black 96-well plates with clear covers (SPL Life Sciences, Korea) at $10^4$ cells/well. Cells were incubated in PBS containing 50 μM DCF-DA for 30 min at 37 °C in the dark. Cell nuclei were stained using 4′,6′-diamidino-2 phenylindole (DAPI; Invitrogen). Cells were incubated with 1 μM DAPI for 1 min at RT in the dark. Fluorescence was then read using a fluorometer with 480 nm excitation and 530 nm emission filters (SpectraMax microplate reader; Molecular Devices, Sunnyvale, CA, USA).

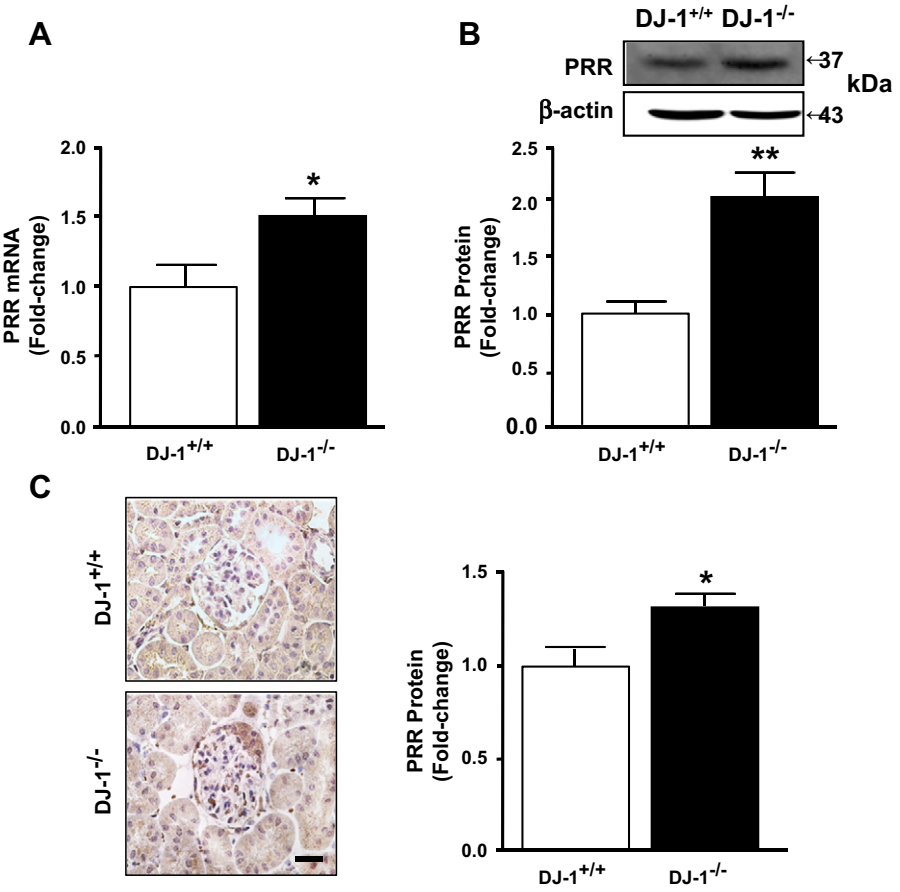


**Fig. 1.** Differential expression of the PRR in the whole kidney from DJ-$1^{-/-}$. (A–B) Expression of PRR mRNA (A) and protein (B) in the kidney tissues was measured by real-time PCR and western blot analysis, respectively. The relative level of PRR mRNA is expressed as fold-change using $2^{-\Delta\Delta Ct}$ values. The level of PRR protein was normalized by β-actin. The density of each band was analyzed using ImageJ software. Data are expressed as mean ± SEM (n = 6). (C) Immunohistochemical analysis of PRR was performed as described in the Materials and methods section. The scale bar represents 25 μm. The graph was obtained from the average intensity analysis of immunohistochemical stains of DAB in the glomeruli of the renal cortex. Data are expressed as mean ± SEM (n = 8). *$P < 0.05$ and **$P < 0.01$ for the differences between DJ-$1^{+/+}$ and DJ-$1^{-/-}$.

### 2.10. Statistical analysis

Data are expressed as mean ± SEM and were analyzed by one-way ANOVA followed by post-hoc Dunnett test for multiple comparisons or unpaired Student's *t* test for comparisons between pairs of groups. A *P* value < 0.05 was considered significant.

## 3. Results

### 3.1. Expression of PRR in the whole kidney from DJ-1$^{-/-}$

To test the influence of PRR in the whole kidney in DJ-1$^{-/-}$, we determine the expression patterns of PRR mRNA and protein in the kidney from DJ-1$^{+/+}$ and DJ-1$^{-/-}$ by performing quantitative real-time PCR and western blotting, respectively. The levels of PRR mRNA in the kidney were increased by about 1.5-fold in DJ-1$^{-/-}$ compared with DJ-1$^{+/+}$ (Fig. 1A). The protein levels of PRR in the kidney were also significantly increased in DJ-1$^{-/-}$ compared with DJ-1$^{+/+}$ (Fig. 1B). Similarly, immunohistochemical analysis showed a stronger PRR expression in the glomeruli from DJ-1$^{-/-}$ than in DJ-1$^{+/+}$ (Fig. 1C).

### 3.2. PRR upregulation by HDAC1-mediated epigenetic modification

Our previous study showed that renal PRR expression was regulated by the modulation of HDAC1 and histone H3 acetylation in hypertensive rats [27]. To investigate a molecular mechanism of PRR upregulation induced by DJ-1 deficiency, we tested HDAC1-mediated epigenetic changes at the PRR promoter in the whole kidney from DJ-1$^{-/-}$. Thus, we predicted 128- and 340-bp CpG islands at the PRR promoter region using the MethPrimer program (http://www.urogene.org/methprimer), and designed the PRR promoter region as shown in Supplementary

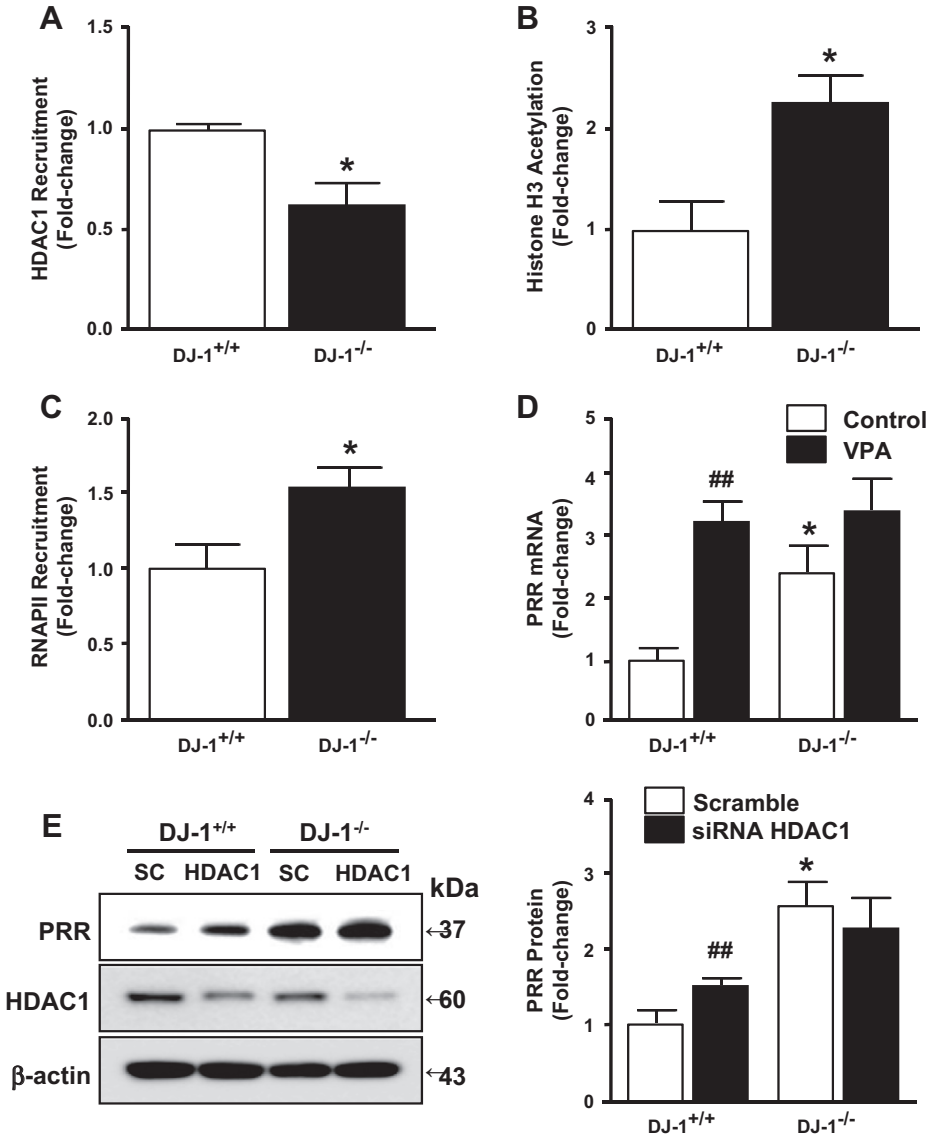


**Fig. 2.** HDAC1-mediated epigenetic regulation in PRR expression. (A–C) Epigenetic regulation in PRR expression in the kidney tissue from DJ-1$^{+/+}$ and DJ-1$^{-/-}$. Chromatin was immunoprecipitated with antibodies against HDAC1 (A), acetyl-histone H3 (B), and RNAPII (C). The PRR promoter region (position −137 to +18) was amplified by quantitative real-time PCR (see Supplementary Fig. S3). The values were normalized by that of input. Data are expressed as mean ± SEM (n = 5). (D) Effects of VPA on the expression of the PRR. After treatment of the mesangial cells isolated from mice with VPA (4 mM) for 24 h, the level of PRR mRNA expression was measured by real-time PCR. The relative level of PRR mRNA is expressed as fold-change using $2^{-\Delta\Delta Ct}$ values. Data are expressed as mean ± SEM (n = 5). (E) Effect of HDAC1 silencing on the expression of the PRR. The mesangial cells were transfected with HDAC1 siRNA (HDAC1) or non-silencing scrambled siRNA (SC). The levels of PRR protein were measured by western blot and were normalized by β-actin (right panel). Transfection efficiency of HDAC1 siRNA was confirmed by using western blot (see Supplementary Fig. S4). Data are expressed as mean ± SEM (n = 6). $^{*}P < 0.05$ for the differences between DJ-1$^{+/+}$ and DJ-1$^{-/-}$. $^{\#\#}P < 0.01$ for the differences between the control and treated groups.

Fig. S3. We next performed a ChIP assay to analyze HDAC1 recruitment, histone H3 acetylation, and RNAPII recruitment at the PRR promoter region (position −137 to +18). HDAC1 recruitment at the PRR promoter region was lower in the kidney tissues from DJ-$1^{-/-}$ compared with DJ-$1^{+/+}$ (Fig. 2A). Histone H3 acetylation and RNAPII recruitment at the PRR promoter region evoked a greater increment in the kidney tissues from DJ-$1^{-/-}$ than in those from DJ-$1^{+/+}$ (Fig. 2B and C). We also investigated the effects of HDAC1 inhibition on PRR expression in mesangial cells isolated from DJ-$1^{+/+}$ and DJ-$1^{-/-}$. In the quiescent state, the expression level of PRR was increased by about 2.5-fold in mesangial cells from DJ-$1^{-/-}$ compared with those from DJ-$1^{+/+}$ (Fig. 2D). Treatment of mesangial cells with VPA (4 mM), a class I HDAC inhibitor, increased the expression level of PRR mRNA in DJ-$1^{+/+}$ cells to a level similar to that in DJ-$1^{-/-}$ cells (Fig. 2D). In addition, HDAC1 knockdown using a siRNA elevated the expression level of PRR protein in DJ-$1^{+/+}$ cells, but not in DJ-$1^{-/-}$ cells (Fig. 2E and Supplementary Fig. S4).

### 3.3. Effects of $H_2O_2$ on histone modification and PRR expression

A typical phenomenon in DJ-1 deficiency represents an increment of cellular ROS level [13]. First, we compared the $H_2O_2$ levels in mesangial cells from DJ-$1^{+/+}$ and DJ-$1^{-/-}$. Production of $H_2O_2$ was significantly increased in mesangial cells from DJ-$1^{-/-}$ compared with those from DJ-$1^{+/+}$ (Fig. 3A). Next, we defined the effect of $H_2O_2$ on histone modification in mesangial cells from DJ-$1^{+/+}$. $H_2O_2$ (10, 30, or 100 μM) treatment increased PRR expression in mesangial cells from DJ-$1^{+/+}$ in a dose-dependent manner (Fig. 3B). $H_2O_2$ also decreased HDAC1 recruitment (Fig. 3C) and elevated histone acetylation and RNAPII recruitment at the PRR promoter in DJ-$1^{+/+}$ cells (Fig. 3D and E).

### 3.4. Effects of NAC, an antioxidant, on histone modification and PRR expression

To confirm the influence of ROS in DJ-$1^{-/-}$, we measured the effect of NAC, an antioxidant reagent, on PRR expression and epigenetic regulation in mesangial cells from DJ-$1^{-/-}$. Treatment of mesangial cells from DJ-$1^{-/-}$ with NAC (1 mM) increased HDAC1 recruitment and decreased histone acetylation at the PRR promoter (Fig. 4A and B) and decreased RNAPII recruitment (Fig. 4C). In addition, NAC also decreased the expression of the PRR in mesangial cells from DJ-$1^{-/-}$ (Fig. 4D).

### 3.5. ERK1/2 activation in response to prorenin in DJ-$1^{-/-}$

ERK1/2 is involved in the signal pathways mediated by PRR activation via AT1 receptor-dependent and -independent pathways [4]. Thus, we compared the level of ERK1/2 phosphorylation in the whole kidneys from DJ-$1^{+/+}$ and DJ-$1^{-/-}$. Phosphorylation of ERK1/2 was greater in the kidney tissues from DJ-$1^{-/-}$ compared with those from DJ-$1^{+/+}$ (Fig. 5A). We also confirmed the phosphorylation levels of ERK1/2 in mesangial cells isolated from the renal cortex. In the quiescent state without any stimulation, the level of ERK1/2 phosphorylation was greater in mesangial cells from DJ-$1^{-/-}$ compared with DJ-$1^{+/+}$ (Fig. 5B), a pattern similar to that seen in the kidney tissues. The elevated resting phosphorylation in DJ-$1^{-/-}$ cells was abolished by the treatment of mH-RP (10 μM), a PRR inhibitor. In contrast, mH-RP did not affect the resting phosphorylation in DJ-$1^{+/+}$ cells. Treatment with prorenin (10 nM) elevated the phosphorylation of ERK1/2 in DJ-$1^{+/+}$ cells, as well as in DJ-$1^{-/-}$ cells (Fig. 5B). ERK1/2 phosphorylation in response to prorenin was attenuated in DJ-$1^{-/-}$ cells, but not in DJ-$1^{+/+}$ cells, treated with mH-RP (10 μM) (Fig. 5B). To determine the

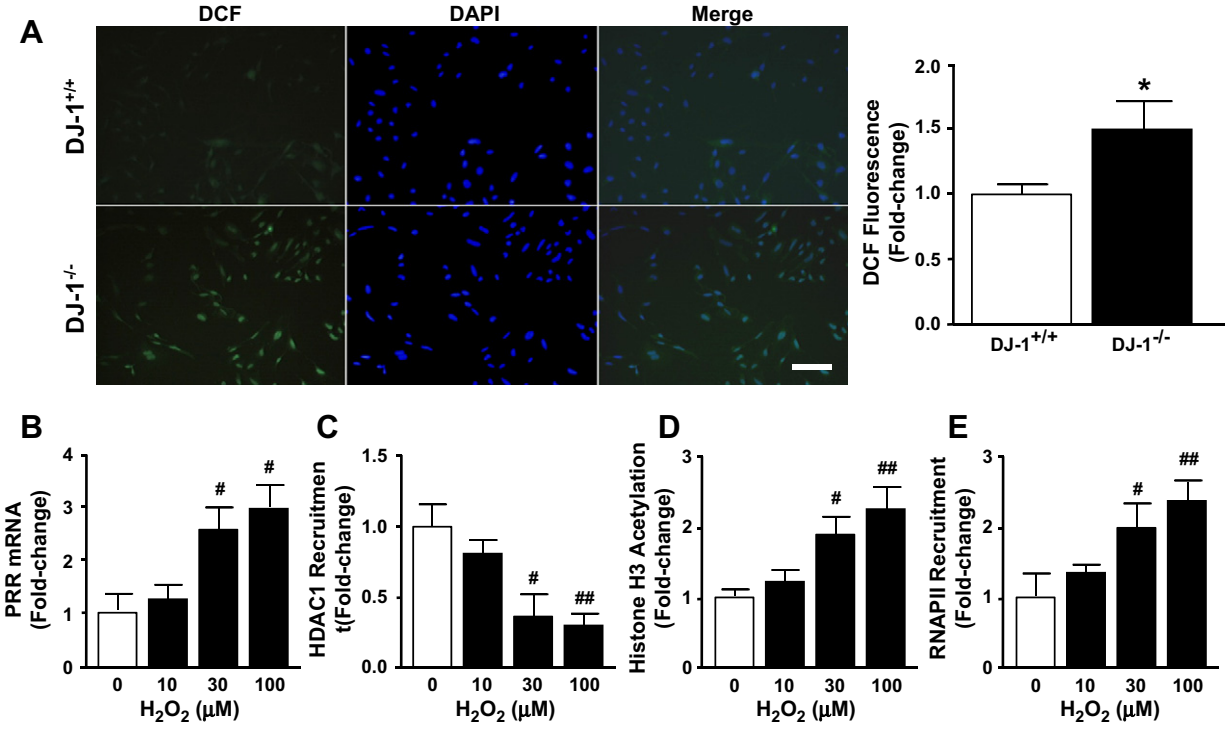


**Fig. 3.** Effects of $H_2O_2$ on histone modification and PRR expression. (A) $H_2O_2$ production in mesangial cells from DJ-$1^{+/+}$ and DJ-$1^{-/-}$. $H_2O_2$ levels were measured by the oxidation of DCF-DA as described in the Materials and methods section. Left panel: The mesangial cells were incubated with DCF-DA (50 μM) in PBS for 30 min at 37 °C. DAPI was used as nuclear stain. The scale bar represents 50 μm. Right panel: Statistical data are expressed as mean ± SEM (n = 6). (B) Effects of $H_2O_2$ on PRR expression. Mesangial cells from DJ-$1^{+/+}$ were treated with $H_2O_2$ for 24 h. The level of PRR mRNA expression was measured by real-time PCR. The relative level of PRR mRNA is expressed as fold-change using $2^{-\Delta\Delta Ct}$ values. Data are expressed as mean ± SEM (n = 4). (C–E) Effects of $H_2O_2$ on the epigenetic modification of PRR promoter in DJ-$1^{+/+}$ mesangial cells. After incubation of mesangial cells from DJ-$1^{+/+}$ with $H_2O_2$ for 24 h, HDAC1 recruitment (C), histone H3 acetylation (D) and RNAPII recruitment (E) at the PRR promoter were measured using the ChIP assay. Data are expressed as mean ± SEM (n = 5). $^{*}P < 0.05$ for the differences between DJ-$1^{+/+}$ and DJ-$1^{-/-}$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ for the differences between the control and treated groups. Statistical differences of data were analyzed by one-way ANOVA followed by the post-hoc Dunnett test.

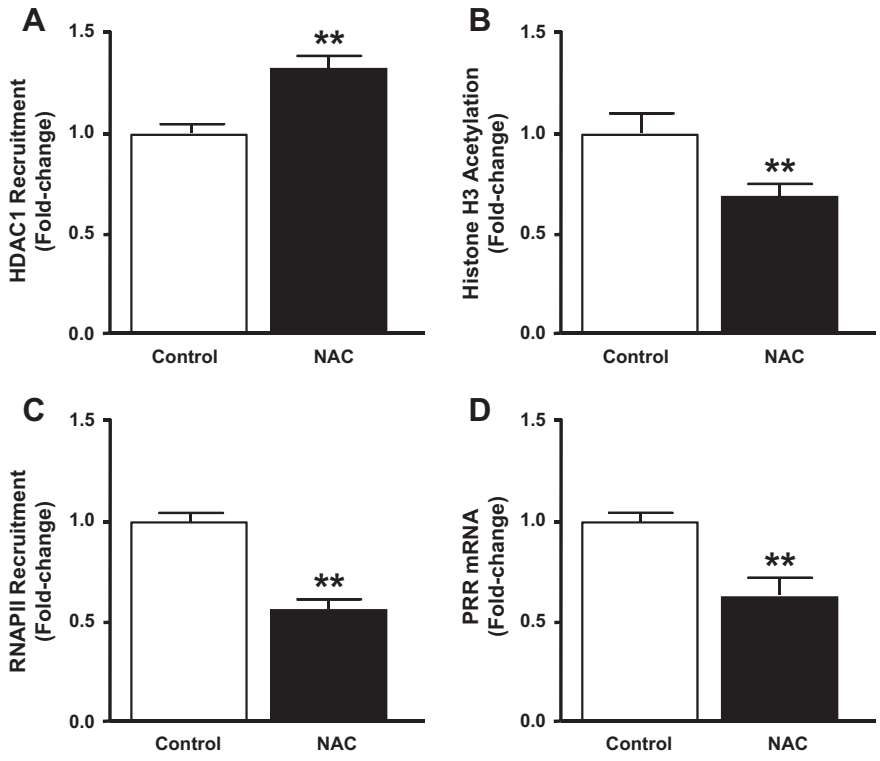


**Fig. 4.** Effect of NAC, an antioxidant, on histone modification and PRR expression. (A–C) Epigenetic modifications in response to NAC. After incubation of mesangial cells from DJ-$1^{-/-}$ with 1 mM NAC for 24 h, HDAC1 recruitment (A), histone H3 acetylation (B), and RNAPII recruitment (C) at the PRR promoter were measured using the ChIP assay (n = 5). (D) Effects of NAC on PRR mRNA expression in mesangial cells. The level of PRR mRNA expression was measured by real-time PCR. The relative level of PRR mRNA is expressed as fold-change using $2^{-\Delta\Delta Ct}$ values. Data are expressed as means ± SEM (n = 5). $^{**}P < 0.01$ for the differences between the control and treated groups.

effect of AT receptor-dependent pathway on ERK1/2 activation, we investigated the effects of losartan (10 μM) and PD123319 (10 μM), inhibitors of AT1 and AT2 receptors, respectively, on the prorenin-induced ERK1/2 phosphorylation. In the presence of losartan and PD123319, prorenin-induced ERK1/2 activation was inhibited in both DJ-$1^{+/+}$ and DJ-$1^{-/-}$ cells (Fig. 5C).

### 3.6. Renal fibrotic gene expression in DJ-$1^{-/-}$

To investigate whether or not DJ-1 is associated with renal fibrosis, we examined the fibrotic gene expression in DJ-$1^{-/-}$. The fibrotic genes such as TGF-β1, Col1a, PAI-1, and Fn1 were increased significantly in the whole kidney tissue from DJ-$1^{-/-}$ compared with DJ-$1^{+/+}$ (Fig. 6A). To determine whether the increase of PPR in DJ-$1^{-/-}$ cells contributes to expression alteration of the fibrotic genes, PRR was knocked down using a siRNA-PRR. Transfection of cells with siRNA-PRR significantly decreased the expression of fibrotic genes in DJ-$1^{-/-}$ cells (Fig. 6B).

## 4. Discussion

In this study, we found that the expression of the renal PRR was higher in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$. Moreover, HDAC1 recruitment at the PRR promoter was diminished and its histone acetylation increased in the kidney tissues from DJ-$1^{-/-}$ compared with DJ-$1^{+/+}$. These results imply that DJ-1 protein participates in the regulation of renal PRR expression via histone H3 acetylation. Acetylation of histone H3 is an important mechanism of gene expression by altering the accessibility of transcription factors to the promoter region [28]. Histone acetylation is regulated by the balance of HAT and HDAC activities [23]. HDACs catalyze the removal of acetyl groups on lysine tails of histone proteins, leading to repression of transcriptional activity [29]. Our results suggest that the activity of HDAC1 was lower in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$ and that this difference in activity was related to the acetylation of histone H3 protein at its promoter following elevated expression of the PRR. HDAC1 inhibition by VPA or HDAC1 siRNA increased the expression of PRR in DJ-$1^{+/+}$ mesangial cells to a level similar to that in DJ-$1^{-/-}$ cells. From these results, it can be assumed that HDAC activity differs between DJ-$1^{+/+}$ and DJ-$1^{-/-}$ despite the similar activity of HAT and that DJ-1 protein regulates the elevation of HDAC1 activity in renal mesangial cells from DJ-$1^{+/+}$.

We also found that the content of ROS, especially $H_2O_2$, was significantly increased in mesangial cells from DJ-$1^{-/-}$ compared with DJ-$1^{+/+}$. A previous study also demonstrated an elevated ROS content in DJ-1-downregulated and -deficient renal cells [14]. Oxidative stress regulates both the binding of transcription factors to their promoters and HDAC activity by altering cysteine residues of HDACs [30,31]. In our study, treatment of mesangial cells with $H_2O_2$ decreased HDAC1 activity and increased not only histone H3 acetylation at the PRR promoter but also the PRR expression. $H_2O_2$ also induced RNAPII recruitment, which plays a predominant role in transcriptional activation following histone acetylation [30,32]. Moreover, treatment with the antioxidant NAC reversed these epigenetic modifications in DJ-$1^{-/-}$ cells. These results suggest that ROS generated in the DJ-1-deficient condition contributes to the upregulation of renal PRR expression by regulating HDAC1 at the PRR promoter. This is the first study, to the best of our knowledge, to show that ROS may regulate the expression of the PRR through epigenetic modulation in renal mesangial cells.

The PRR is implicated in the development of renal damage as a part of the RAS [33,34]. The overexpression of the PRR in transgenic animals generates glomerulosclerosis and proteinuria [9,10]. The administration of a PRR-inhibitory peptide attenuates renal damage, including glomerulosclerosis, proteinuria, and glomerular fibrosis [35,36]. Reversely, PRR expression is upregulated in a variety of diseases, such as

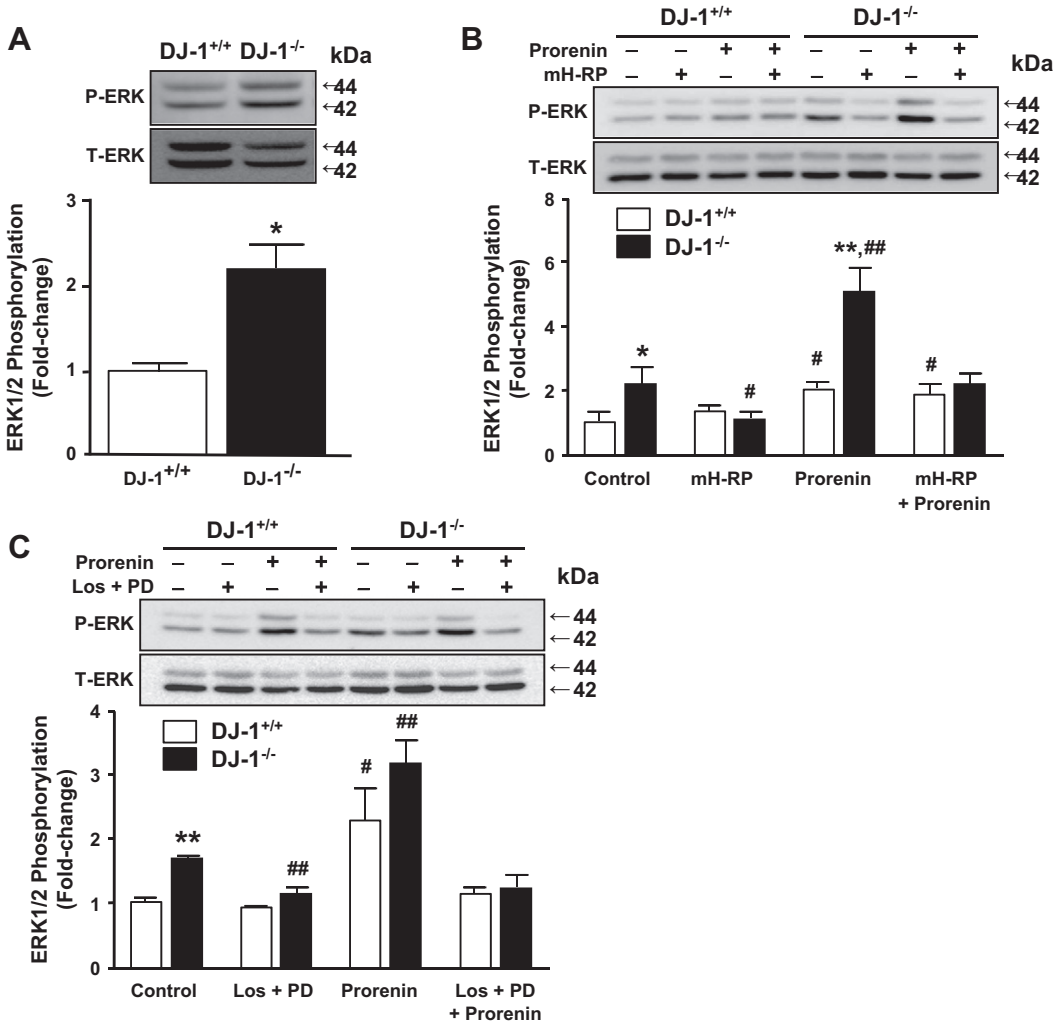


**Fig. 5.** ERK1/2 activation in response to prorenin in DJ-1$^{-/-}$. (A) The levels of ERK1/2 phosphorylation in the whole kidney. The ERK1/2 phosphorylation was measured by western blot analysis (n = 6). (B) Effect of mH-RP on prorenin-induced ERK1/2 phosphorylation in mesangial cells. After preincubation with mH-RP (10 μM) for 30 min, mesangial cells were stimulated with prorenin (10 nM) for 10 min (n = 6). (C) Effects of AT receptor inhibitors on ERK1/2 phosphorylation in mesangial cells. After preincubation with losartan (Los, 10 μM) and PD123319 (PD, 10 μM) for 30 min, the cells were stimulated with prorenin (10 nM) in the presence of both losartan and PD123319 for 10 min (n = 4). The level of ERK1/2 phosphorylation (P-ERK) was normalized by total ERK1/2 (T-ERK). The density of each band was analyzed using ImageJ software. Data are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ for the differences between DJ-1$^{+/+}$ and DJ-1$^{-/-}$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ for the differences between the control and treated groups.

renal fibrosis, hypertension, or diabetes [27,36–39]. These results imply that various diseases are interacted with the expression of PRR through pathophysiological process of redox system.

Moreover, we found in this study that ERK1/2 activation was increased in the renal tissues and mesangial cells from DJ-1$^{-/-}$ compared with DJ-1$^{+/+}$. Prorenin-treated mesangial cells exhibited increased ERK1/2 activation, which was inhibited in treatment with mH-RP. This mH-RP also attenuated the resting activation of ERK1/2 in DJ-1$^{-/-}$ mesangial cells, which reached a level similar to the resting of DJ-1$^{+/+}$. Although other studies showed controversial results in vivo and in vitro data using mH-RP [40,41], our results revealed an inhibitory effect of mH-RP on ERK1/2 activation in mesangial cells. In addition, the ERK1/2 activation in response to prorenin was abolished by AT1 and AT2 receptor blockers in DJ-1$^{+/+}$ and DJ-1$^{-/-}$ mesangial cells. From these results, it can be assumed that elevated PRR may activate its signal pathway in Ang II/AT receptor-dependent manner in DJ-1-deficient state. The expression of fibrotic genes is involved in the development of renal fibrosis and its expression is mediated by ERK1/2 activity [42]. PRR activation stimulated the expression of fibrotic genes by regulating ERK1/2 activity in AT1 receptor-dependent or independent manners [6–8,43]. Here, we showed that the expression of fibrotic genes was significantly elevated in DJ-1$^{-/-}$ compared with DJ-1$^{+/+}$ and that was accompanied by alteration in the pattern of PRR expression. Moreover, transfection of cells with siRNA-PRR significantly decreased the expression of fibrotic genes in DJ-1$^{-/-}$ cells. Therefore, it may be assumed that the elevated expression of the PRR leads to an increase in ERK1/2 activity-dependent renal fibrosis from DJ-1$^{-/-}$.

Neuronal, vascular, and renal disorders are thought to be regulated by an imbalance in cellular redox systems [44]. ROS is involved in cellular damage in the kidney, and treatment with antioxidants or overexpression of antioxidant proteins prevents renal damage [45]. DJ-1 acts as a ROS scavenger and reduces the level of oxidative stress in a variety of cells [13]. We and others have demonstrated previously that DJ-1 deficiency contributes to increase of blood pressure in age-dependent manner [14,18]. These results suggest that DJ-1 can participate in oxidative stress-related pathogenesis in a variety of cellular systems and that DJ-1 may act as a key protein in the development of various diseases, including renal dysfunction. Therefore, we suggest that DJ-1 and PRR expressions could have an inversely regulated relationship and that this information may be useful in the development of treatments for renal diseases and other oxidative stress-related disorders.

In summary, the level of PRR in the kidney was significantly increased in DJ-1$^{-/-}$ compared with DJ-1$^{+/+}$. Histone H3 acetylation and RNAPII recruitment at the PRR promoter were higher in DJ-1$^{-/-}$

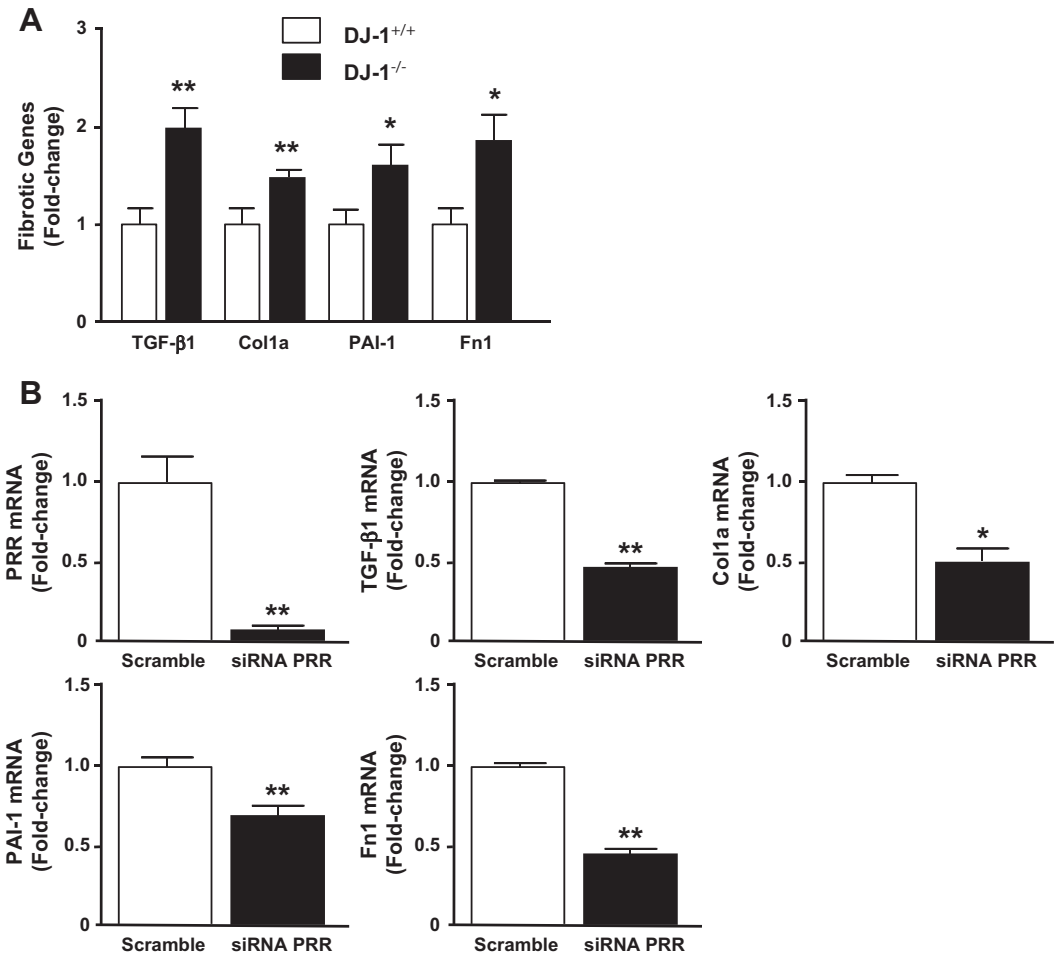


**Fig. 6.** Expression of fibrotic genes in DJ-$1^{-/-}$. (A) The mRNA expression levels of fibrotic genes (TGF-β1, Colla, PAI-1 and Fn1) in the whole kidney tissues (n = 6). (B) Effect of PRR silencing on the expression of fibrotic genes. The DJ-$1^{-/-}$ mesangial cells were transfected with PRR siRNA or non-silencing scrambled siRNA (n = 4). Level of mRNA expression was measured by real-time PCR. The relative level of mRNA is expressed as fold-change using $2^{-\Delta\Delta Ct}$ values. Data are expressed as mean ± SEM. *$P < 0.05$ and **$P < 0.01$ for the differences between DJ-$1^{+/+}$ and DJ-$1^{-/-}$.

than in DJ-$1^{+/+}$ kidney tissues. HDAC1 inhibitions restored PRR expression in mesangial cells from DJ-$1^{+/+}$. These changes in PRR expression and epigenetic modification in DJ-$1^{-/-}$ cells could be induced by treatment with $H_2O_2$ and could be reversed by addition of an antioxidant reagent. ERK1/2 activation in response to prorenin was greater in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$ cells and this was inhibited by a PRR-inhibitory peptide and by AT1 and AT2 receptor inhibitors. The expression of fibrotic genes was greater in DJ-$1^{-/-}$ than in DJ-$1^{+/+}$ cells and was attenuated in DJ-$1^{-/-}$ cells subjected to PRR-siRNA transfection. These data suggest that DJ-1 deletion may contribute to the acceleration of renal damage, through $H_2O_2$-mediated epigenetic regulation of renal PRR expression.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2014.11.017.

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