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DJ-1 cooperates with PYCR1 in cell protection against oxidative stress

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ABSTRACT

DJ-1, a product of the *DJ-1/PARK7* gene, has been suggested to play various functions involved in transcriptional regulation, protease activity, anti-oxidative stress activity, and regulation of mitochondrial complex I. Such a variety of functions of DJ-1 are supposed to be realized through interactions with different partner proteins. Among the candidates for DJ-1-partner proteins detected in TOF-MAS analyses of the cellular proteins co-immunoprecipitated with DJ-1, we focused here pyrroline-5-carboxylate reductase 1, PYCR1, a final key enzyme for proline biosynthesis. DJ-1 directly bound to PYCR1 *in vivo* and *in vitro*. DJ-1 and PYCR1 colocalized in mitochondria, and both were suggested to be involved in regulation of mitochondrial membrane potential, but differently. DJ-1 enhanced the enzymatic activity of PYCR1 *in vitro*. The cells knocked down for DJ-1 and PYCR1 showed lower viability under oxidative stress conditions. No additive nor synergistic results were obtained for the cells that had been knocked down for both DJ-1 and PYCR1, suggesting that DJ-1 and PYCR1 are on the same pathway of anti-oxidative stress protection of the cells.

1. Introduction

The *DJ-1* gene was originally cloned by our group as an oncogene that transforms NIH3T3 cells cooperatively with activated H-*ras* [1]. The *DJ-1* gene later turned out to be identical to *PARK7*, one of the causative genes for familial Parkinson's disease, the second most common neurodegenerative disease [2]. There are 18 *PARK* genes, so far reported as causative genes for familial Parkinson's disease [3], DJ-1 and PINK1 among them has been reported to be involved in responses to anti-oxidative stress, which is considered a major trigger of Parkinson's disease pathogenesis [4,5]. Dopaminergic neuronal cell death in the substantia nigra of the midbrain is typically observed in Parkinson's disease patients, and the depression of dopamine yields movement disorders. DJ-1, the product of *DJ-1/PARK7*, is a small protein comprised of 186 amino acids and is a multi-functional protein involved in regulation of transcriptions and mitochondrial complex 1 and modulation of signaling pathways [4,5]. Such functions are suggested to diminish oxidative stress and to protect neuronal cells from undesirable cell death [4,5]. In the brains of both familial and sporadic Parkinson's disease patients, exceeding the amount of hyper-oxidized form of DJ-1 has been observed. It is thus suggested that a hyper-oxidized form of DJ-1 is abnormal in terms of its functions and accounts for the onset of Parkinson's disease. A variety of functions of DJ-1 is supposed to be demonstrated through interaction with different proteins. Identification and characterization of specific partner proteins associating with DJ-1 is crucial to understand the DJ-1-related onset mechanism of Parkinson's disease. Cellular proteins were precipitated with anti-DJ-1 antibodies, and the proteins found in the precipitates were assumed to form complexes with DJ-1 and analyzed. Among a lot of candidate proteins for DJ-1 partners, we focused on PYCR1 in this study.

PYCR1 is a house keeping enzyme required for the final step of proline biosynthesis from microorganisms to higher plants and animals. PYCR1 comprises 319 amino acids and localizes to mitochondria. A number of studies have been reported about the involvement of PYCR1 in *cutis laxa*, a group of rare connective tissue disorders [6]. Mutations within the PYCR1 gene are often detected in autosomal recessive patients of *cutis laxa*, whose skin shows a significant decrease in elastin, a major component of extracellular matrix. Correlation between PYCR1 and matrix metalloproteinases (MMPs) has been suggested [7]. Proline is suggested to have anti-apoptotic and anti-oxidant activities [8],

Abbreviations: PYCR1, pyrroline-5-carboxylate reductase 1; P5C, pyrroline-5-carboxylate; POX, proline oxidase; ROS, reactive oxygen species.

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while P5C, the substrate of PYCR1, has been reported for pro-apoptotic activity and growth inhibition [9]. PYCR1 may therefore quantitatively control the balance between endogenous substances for pro-apoptotic and those for anti-apoptotic directions, possibly in response to stress conditions in cells.

2. Materials and methods

2.1. Cell culture and knockdown of DJ-1 and PYCR1

Human HeLa cells and 293T cells were purchased from American Tissue culture collection (ATCC). DJ-1-knockout (DJ-1(−/−)) mouse cells that had been immortalized with SV40 T-antigen were described previously [10]. Two cell lines were established from the DJ-1(−/−) cells transfected with the pcDNA3-derived expression vector for FLAG-DJ-1 or the empty vector, together with that for the hygromycin B-resistant gene and cultured in the presence of 400 μg/ml hygromycin B. About 3–4 weeks after transfection, a hygromycin B-resistant cell line expressing FLAG-DJ-1 was isolated and designated as DJ-1(−/−)-F-DJ-1 cells. Another hygromycin B-resistant cell line carrying the empty vector was isolated and designated as DJ-1(−/−)-empty cells. The nucleotide sequences for siRNA targeting human *DJ-1* were as follows: 5′-UGGAGACGGUCAUCCCUGUdTdT-3′ (upper strand) and 3′-dTdTACCUCUGCCAGUAGGGACA-5′ (lower strand). The siRNA SASI_Hs01_00166307 (Sigma) was used as the siRNA for the *PYCR1* gene. Non-specific siRNA for control experiments (Allstar siRNA) was purchased from Qiagen (Valencia, USA). The siRNAs were transfected into cells using RNAiMAX (Invitrogen, USA) according to the supplier's protocol. T-REx™-293 pRNA-H1-tet-hygro-hDJ-1, a DJ-1 knockdown-inducible cell line, was established by use of T-REx™-293 cells and T-REx™ System (Invitrogen, Life Technologies, USA).

2.2. TOF-MAS analyses

The proteins extracted from HeLa cells and immunoprecipitated with anti-DJ-1 antibodies or normal IgG were separated on a 10% polyacrylamide gel and visualized by staining with silver. The protein bands specifically observed in the precipitates with three different anti-DJ-1 antibodies but not in the precipitate with IgG were clipped. The antibodies used were an anti-DJ-1 polyclonal antibody (NB 300-270, Novus Biologicals, USA), an affinity-purified rabbit anti-DJ-1 polyclonal antibody [1] and another anti-DJ-1 antibody prepared with the peptide of 15 amino acids at the C-terminus of DJ-1 (designated as α-DJ-1(A), α-DJ-1(B) and α-DJ-1(C) in Fig. 1A, respectively). The proteins in the clipped gel were subjected to TOF-MAS analyses as described previously [11].

2.3. Western blotting and antibodies

The cells were lysed as described [12] and centrifuged. The proteins in the supernatant were then separated on a 12.5% polyacrylamide gel and subjected to Western blotting with respective antibodies. Proteins on the membrane were reacted with an IRDye 800- (Rockland, USA) or Alexa Fluor 680-conjugated secondary antibody (Molecular Probes, USA) and visualized by using an infrared imaging system (Odyssey, Li-COR, Lincoln, USA). Antibodies used were as follows: a monoclonal anti-DJ-1 (MBL, USA), anti-PYCR1 (Preteintech, USA), anti-FLAG (M2, Sigma–Aldrich, USA), anti-HA (Santa Cruz, USA), anti-actin (Chemicon, USA) antibodies. The rabbit anti-DJ-1 antibody for immunoprecipitation was prepared by us as described previously [1].

2.4. In vivo co-immunoprecipitation assay

Proteins were extracted from cells using a buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl and 0.5% Triton-X100, and immunoprecipitated with an anti-FLAG antibody, anti-DJ-1 antibody, or mouse or rabbit normal IgG (MBL, USA), and the precipitates were analyzed by Western blotting using an anti-HA, anti-FLAG, anti-PYCR1, or anti-DJ-1 antibody described above.

2.5. Pull-down assay

^{35}S-labeled FLAG-PYCR1 was synthesized *in vitro* using the reticulocyte lysate of the TNT transcription-translation coupled system (Promega, USA). Labeled proteins were mixed with GST or GST-DJ-1 that had been expressed in and prepared from *Escherichia coli* at 4 °C for 60 min in a buffer containing 150 mM NaCl, 5 mM EDTA, 50 mM Tris (pH 7.5), 0.05% bovine serum albumin, 0.1% Nonidet P-40 (NP-40) and applied on glutathione Sepharose beads (GE Healthcare, Japan). After washing the resin with the same buffer, the proteins bound to the resin were separated in a 10% polyacrylamide gel containing SDS and visualized by fluorography.

2.6. Indirect immunofluorescence

Cells were fixed with 4% paraformaldehyde and reacted with rabbit anti-DJ-1 polyclonal and mouse anti-PYCR1 antibodies. The cells were also stained with MitoTracker Red (Life Technologies, USA). The cells were then reacted with a rhodamine-conjugated anti-rabbit IgG or an FITC-conjugated anti-mouse IgG and observed under a Bio-imaging system (OLYMPUS, FSV100, Japan).

2.7. Enzymatic activity assays for PYCR1

The DJ-1(−/−)-F-DJ-1 cells or DJ-1(−/−)-empty cells were collected, suspended in a buffer containing 100 mM potassium phosphate (pH 7.0), 150 mM NaCl and protease inhibitor cocktail (Sigma–Aldrich, USA) and centrifuged at 23,180*g* for 10 min at 4 °C after sonication. GST and GST-PYCR1 were bacterially expressed and purified after treatment with PreScission Protease (GE Healthcare, USA). Fifty micrograms proteins of cell lysates from either cell line were added to 0.1 μg of GST or GST-PYCR1. After 60 min on ice, the enzymatic reaction of PYCR1 was initialized by adding the buffer containing 100 mM potassium phosphate (pH 7.0), 0.1 mM NADH (Wako Pure Chemicals, Japan) and 1 mM P5C. P5C was chemically synthesized by M.A. and S.S. in authors. After 30 min incubation at room temperature, NADH consumption was estimated by changes in absorption at 340 nm. The NADH degradation in the mixture without P5C was also assayed as controls.

2.8. Cell viability assay

Two-thousand of HeLa cells were seeded in a well of a 96-well plate and cultured for 14–18 h. The cells were then transfected with siRNA for DJ-1 or PYCR1 or with control siRNA. At 64 h after transfection, the cells were treated with various concentrations of hydrogen peroxide for 3 h, and their viability was then measured by using a cell counting kit 8 (DOJINDO, Japan). As for Tet-DJ-1-knockdown cells or its host cells [12], 6,000 cells were seeded in a well of a 96-well plate and cultured for 12–16 h. The cells were then transfected with pcDNA3-POX-FLAG-6xHis or pcDNA3 by the calcium phosphate method [13] and cultured for another 8 h. The cells were further cultured in the presence or absence of 1 μg/ml doxycycline for 4 days and the viabilities were measured as described above.

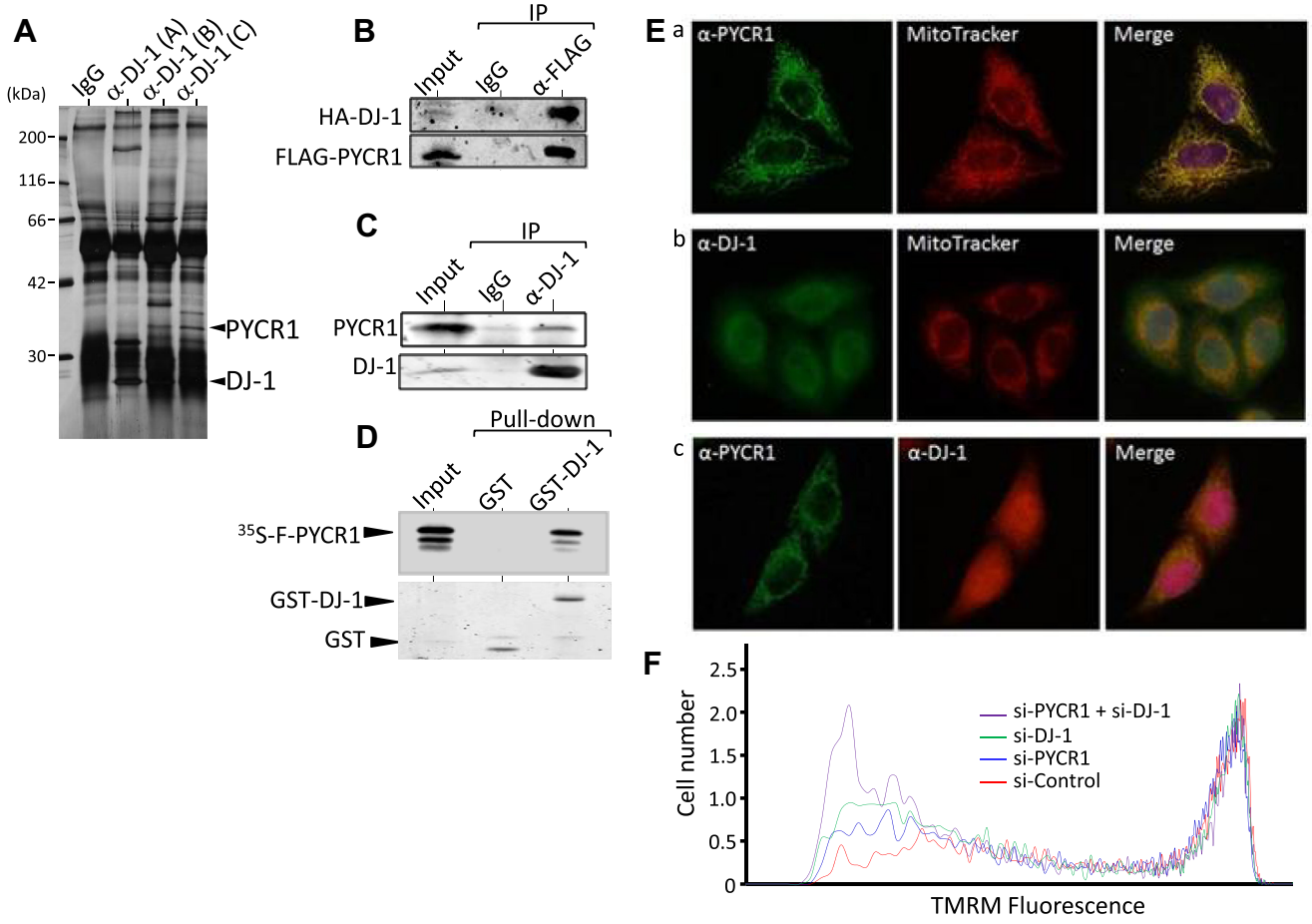

Fig. 1. Association of DJ-1 with PYCR1. (A) Three different anti-DJ-1 antibodies (A–C) and control IgG were used for immunoprecipitation experiments using cell extracts prepared from HeLa cells. The precipitated proteins were then separated on an SDS-containing polyacrylamide gel and stained with Silver Stain MS Kit. The bands corresponding to PYCR1 and DJ-1 identified by TOF-MS analysis are indicated by arrowheads. (B) Proteins were extracted from human 293T cells transfected with expression vectors for HA-DJ-1 and FLAG-PYCR1, and immunoprecipitated (IP) with an anti-FLAG antibody or IgG. The precipitates were analyzed by Western blotting using anti-HA and anti-FLAG antibodies. (C) Proteins extracted from human HeLa cells were immunoprecipitated (IP) with a rabbit anti-DJ-1 polyclonal antibody or IgG, and the precipitates were analyzed by Western blotting using anti-PYCR1 and mouse anti-DJ-1 monoclonal antibodies. (D) GST or GST-DJ-1 prepared from *E.coli* was mixed with ^{35}S-labeled FLAG-PYCR1 and subjected to pull-down assays. Upper panel, ^{35}S-labeled FLAG-PYCR1 and an input sample were visualized by fluorography. Lower panel, recombinant GST and GST-DJ-1 used in assays were electrophoresed and stained with coomassie brilliant blue. (E) HeLa cells were immunostained with an anti-PYCR1 or anti-DJ-1 antibody (rows a and b in left panel, respectively) and then reacted with an FITC-conjugated secondary antibody, followed by staining with MitoTracker Red (rows a and b in center panels). In cells immunostained with both anti-PYCR1 and anti-DJ-1 antibodies, the target proteins were visualized with FITC- or rhodamine-conjugated secondary antibodies (row c in left and central panels, respectively). (F) Mitochondrial membrane potential was measured as TMRM fluorescence as described in 'Materials and methods'. HeLa cells that had been transfected with siRNA for DJ-1 or PYCR1 or with control siRNA were incubated with TMRM and their fluorescence was measured. The cell number per 10,000 cells in each fluorescence intensity is plotted.

2.9. Measurement of mitochondrial membrane potential

Six-thousand of HeLa cells were seeded in a well of 48-well plate and cultured for 14–18 h. The cells were then transfected with siRNA for DJ-1or PYCR1 or with control siRNA. At 64 h after transfection, the cells were incubated with 250 nM TMRM (ImmunoChemistry Technologies, LLC, USA) for 20 min at 37 °C in a CO_2. The cells were then washed twice with PBS(−) and trypsinized. After centrifuging at 900*g* for 3 min, the cells were suspended in 50 μl of PBS(−) and 20 μl of the cell suspension were injected into Tali® Cellular Analysis Slides (Invitrogen, Life Technologies, USA). Mitochondrial membrane potential of the cells was measured as TMRM fluorescence using Tali® Image-Based Cytometer (Invitrogen, Life Technologies, USA).

2.10. Statistical analyses

Data are expressed as mean ± SE. Statistical analyses were performed using analysis of variance (one-way ANOVA) followed by unpaired Student's *t*-test. For comparison of multiple samples, the Tukey–Kramer test was used.

3. Results and discussion

3.1. Association and co-localization of DJ-1 with PYCR1

Proteins extracted from HeLa cells were subjected to immunoprecipitation using three different anti-DJ-1 antibodies, and the proteins recovered from the precipitates were separated by SDS-gel electrophoresis. The band due to the proteins precipitated with anti-DJ-1 antibodies but not with control IgG were clipped and analyzed by TOF-MAS. Pyrroline-5-carboxylate reductase 1, PYCR1, was thus identified as associating partners of DJ-1 (Fig. 1A). The association between DJ-1 and PYCR1 was confirmed by several experiments. The expression vectors for HA-tagged DJ-1 and FLAG-tagged PYCR1 were transfected into 293T cells. The proteins extracted from the cells were immunoprecipitated with an anti-FLAG antibody, or with normal IgG, and analyzed. Both FLAG-

PYCR1 and HA-DJ-1 were detected in the precipitate with the anti-FLAG antibody (Fig. 1B). FLAG-PYCR1 and HA-DJ-1 were thus suggested to be in the same protein complex in the transfected 293T cells. The cell extract prepared from HeLa cells were immunoprecipitated using the anti-DJ-1 polyclonal antibody or normal IgG, and the proteins in the precipitates were electrophoresed and blotted with the monoclonal anti-DJ-1 antibody or an anti-PYCR1 antibody. As shown in Fig. 1C, not only DJ-1 but also PYCR1 were detected in the precipitate with the anti-DJ-1 polyclonal antibody, but neither protein was observed in the precipitates with IgG. The results indicate the association of endogenous DJ-1 with PYCR1 in cells. Furthermore, GST-DJ-1 was incubated with ^{35}S-labeled FLAG-PYCR1 that had been synthesized *in vitro*, and the proteins precipitated with an anti-GST antibody were analyzed. The ^{35}S-labeled FLAG-PYCR1 was detected with GST-DJ-1, but not with GST alone (Fig. 1D). The results indicate that DJ-1 directly interacted with PYCR1 even in the absence of other cellular proteins.

When HeLa cells were immunostained with an anti-PYCR1 or anti-DJ-1 antibody, a majority of PYCR1 was detected in mitochondria (Fig. 1E-a) as reported previously [6]. DJ-1 was ubiquitously distributed in the cells including mitochondria (Fig. 1E-b), which is consistent with the former report [4]. In the cells stained with both antibodies against PYCR1 and DJ-1, fluorescences due to PYCR1 (green) and DJ-1 (red) were merged into yellow in mitochondria (Fig. 1E-c), indicating that DJ-1 is colocalized with and directly interacted with PYCR1 in mitochondria. Mitochondrial membrane potential, the decrease or loss of which is a characteristic for apoptosis, was measured in HeLa cells with or without introduction of siRNA for PYCR1 and DJ-1. As shown in Fig. 1F, knockdown of either DJ-1 or PYCR1 induced significant increase of cells losing mitochondrial membrane potential. When the expressions of both DJ-1 and PYCR1 were knocked down, additional number of the cells showed decrease/loss of the potential. The results suggest that DJ-1 and PYCR1 regulate mitochondrial membrane potential, but in different pathways.

3.2. Effect of DJ-1 on enzymatic activity of PYCR1

The results above suggest that DJ-1 and PYCR1 mutually affect their functions through direct association. The enzymatic activity of PYCR1 was therefore examined *in vitro* in the presence or absence of DJ-1. PYCR1 was bacterially prepared and purified after treatment with PreScission Protease. As a DJ-1 source, cell lysates were prepared from DJ-1(−/−) cell-derived cell lines harboring an expression vector for FLAG-DJ-1 or an empty vector. The cell lysates prepared from either cell line were added to PYCR1 or GST alone, and PYCR1 enzymatic activity was examined by measuring NADH consumption. As shown in Fig. 2A, the activity of PYCR1 was increased in the presence of DJ-1, suggesting that DJ-1 promotes PYCR1 enzymatic activity via direct association. The NADH degradation was also observed in the mixture without P5C, probably due to various NADH-dependent enzymes, including PYCR1derived from cell lysates. The NADH consumption in reactions was thus represented by subtraction of the consumption obtained in the absence of P5C as background. A slight activity detected for GST and the lysate of DJ-1(−/−)-F-DJ-1 cells might be due to activation by DJ-1 of endogenous PYCR1, but showed no significant difference to that with the lysate of DJ-1(−/−)-empty cells. The expression levels of endogenous PYCR1 in two cell lines were comparable (Fig. 2B). The increase of enzymatic activity observed in the reaction with the DJ-1-containing cell lysate was thus unlikely due to the difference in endogenous PYCR1 activity in the cell lysates added.

3.3. Cell protection activities of DJ-1 and PYCR1 against oxidative stress

Promotion of anti-oxidative stress reactions is of major functions of DJ-1. DJ-1 absorbs reactive oxygen species (ROS) by self-oxidation of three cysteine residues at 46, 53 and 106. Proline, a product of PYCR1-dependent reaction, has also been suggested to be anti-apoptotic, while P5C, a substrate of PYCR1, has been reported to have pro-apoptotic activity [8]. To verify physiological roles of interaction between DJ-1 and PYCR1, cell viability under oxidative stress conditions was examined in DJ-1- and PYCR1-knockdown cells. HeLa cells were transfected with siRNA for DJ-1 or PYCR1 or with control siRNA. At 64 h after transfection, cells were incubated with various concentrations of H_2O_2 for 3 h and their viabilities were measured by an MTT assay. As shown in Fig. 3A, the cells transfected with siRNA for DJ-1 were more vulnerable to the oxidative stress conditions than were those transfected with control siRNA, indicating that DJ-1 has anti-oxidative stress

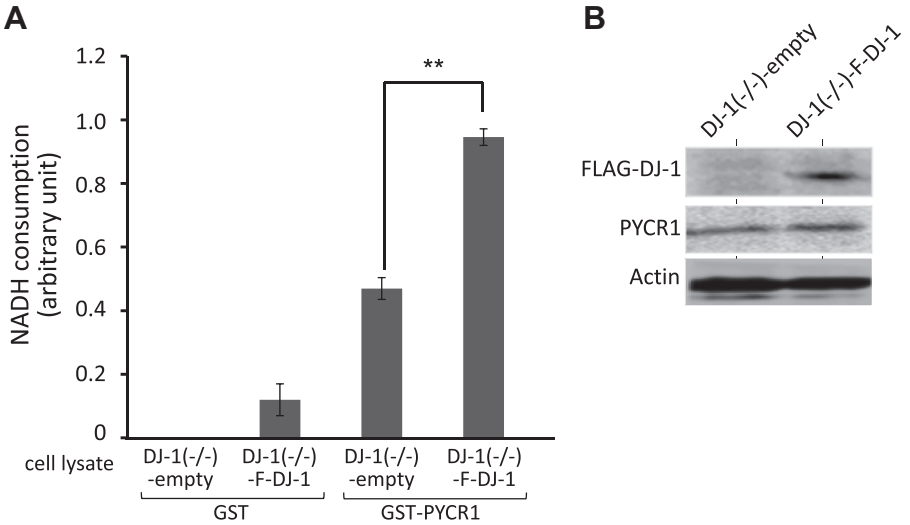

Fig. 2. Effect of DJ-1 on PYCR1 enzyme activity. (A) PYCR1 activity was measured using recombinant GST-PYCR1 or GST purified from *E. coli* in the presence or absence of cell lysates prepared from DJ-1(−/−) cell lines harboring FLAG-tagged wild-type DJ-1 (DJ-1(−/−)-F-DJ-1) or empty vector (DJ-1(−/−)-empty). Statistical analyses were carried out using the Tukey–Kramer test. Number of experiments (n) is 5 ($^{**}P < 0.01$). (B) Proteins extracted from DJ-1(−/−)-F-DJ-1 and DJ-1(−/−)-empty cells were analyzed by Western blotting using anti-PYCR1, rabbit anti-DJ-1 and anti-actin antibodies.

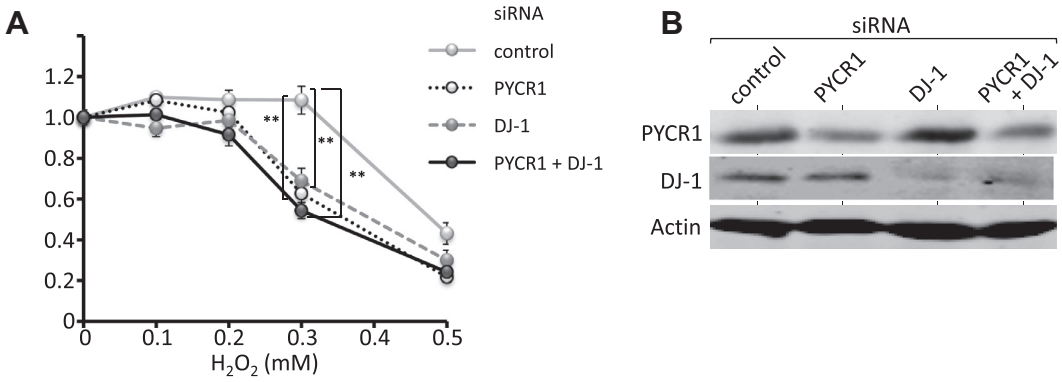

Fig. 3. Effect of PYCR1- and DJ-1-knockdown on H_2O_2-induced cell death. (A) HeLa cells were transfected with siRNAs for DJ-1 and PYCR1 or with control siRNA. At 64 h after transfection, various concentrations of H_2O_2 were added to cells for 3 h and cell viability was measured by an MTT assay as described in 'Materials and methods'. Data of 5 independent experiments were statistically examined by the Tukey–Kramer test ($^{*}P < 0.05$, $^{**}P < 0.01$). (B) Proteins extracted from transfected cells at 64 h as described in the legend for Fig. 3A were analyzed by Western blotting using anti-PYCR1, anti-DJ-1 and anti-actin antibodies.

functions. Similarly, the introduction of siRNA for PYCR1 decreased cell viability by oxidative stress, suggesting that PYCR participates in cellular protection against oxidative stress. When both the siRNAs for DJ-1 and PYCR1 were cotransfected into the cells, the decreased level of cell viability was not significantly enhanced compared to that obtained with respective siRNA for either protein. The results suggest that DJ-1 and PYCR1 are involved in the same pathway/mechanism of cell protection against oxidative stress. As for the role of PYCR1 in anti-oxidative stress protection, proline oxidase (POX), which catalyzes the reverse reaction promoted by PYCR1, should be considered for commitment. POX converts anti-apoptotic proline into proapoptotic P5C, and has thus been suggested to decrease cell viability via P5C induction [9]. Several reports have recently shown that POX induces ROS production and affect decrease in cell viability due to POX [14,15]. To examine the effect of POX on the cell protection activity of DJ-1 against oxidative stress, overexpression of POX was introduced to 293-derived cells where DJ-1 knockdown can be induced by addition of doxycycline, Viability of parental 293 cells was slightly decreased by introduction of additional POX expression both in the presence or absence of doxycycline (Fig. 4A. left panel), suggesting that POX negatively affects the cell viability, possibly by increase of proapoptotic P5C and/or by induction of intracellular ROS. Doxycycline itself did not bring significant difference in the cell numbers of survival in host 293 cells transfected with either the POX-FLAG-6xHis expression vector or an empty vector. When the DJ-1

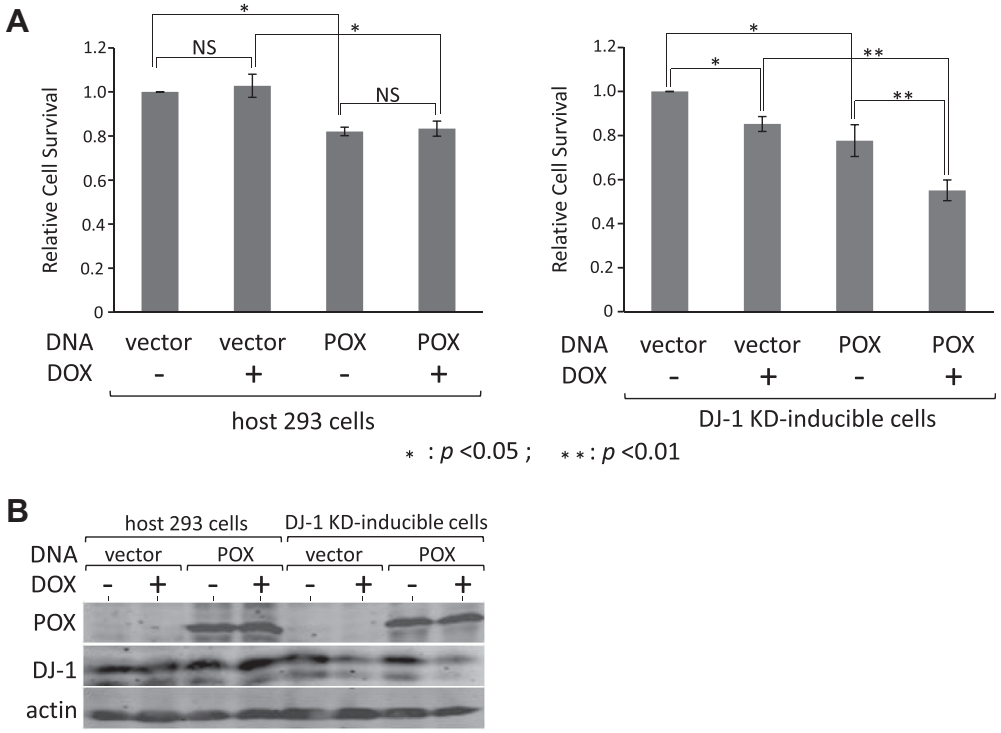

Fig. 4. Effect of additional POX expression and DJ-1-knockdown on H_2O_2-induced cell death. (A) DJ-1 knockdown-inducible cells (DJ-1 KD-inducible cells, T-REx™ -293 pRNA-H1-tet-hygro-hDJ-1 cells, right panel) and parental T-REx™ -293 cells (host 293 cells, left panel) were transfected with the expression vector for FLAG-POX-FLAG-6xHis or vector alone and cultured for 8 h. The cells were further cultured in the presence or absence of 1 μg/ml doxycycline (DOX) for 4 days. Cell viability was measured as described in 'Materials and methods'. Statistical analyses were carried out using the Tukey–Kramer test. Number of experiments (n) is 6. NS: not significant ($^{*}P < 0.05$, $^{**}P < 0.01$). (B) Proteins extracted from the cells transfected and treated as in A were analyzed by Western blotting using anti-FLAG, anti-DJ-1 and anti-actin antibodies.

knockdown was induced by doxycycline, viability of the cells transfected with an empty vector DNA was decreased (Fig. 4A, right panel). The cell number of survival decreased by additional POX expression was further decreased by DJ-1 knockdown. The results suggest that DJ-1 has cell protective functions against impairing effects due to POX overexpression. DJ-1 may contribute to keep good balance between proapoptotic P5C and antiapoptotic proline by regulating PYCR1 activity via direct interaction. DJ-1 may also absorb intracellular ROS induced by POX.

We reported here that the interaction of DJ-1 with PYCR1 is involved in cell protection against oxidative stress. DJ-1 enhanced the enzymatic activity of PYCR1 *in vitro* and is thus suggested to promote the production of antiapoptotic proline via activation of PYCR1 *in vivo*. Proline is therefore a promising candidate for therapeutic medicine to alleviate the symptoms and progression of Parkinson's disease due to dysfunction of DJ-1. P5C, the substrate of PYCR1, on the other hand, has proapoptotic activity. Varying the activity dependent on its oxidative states [5], DJ-1 may function as a sorter balancing antiapoptotic proline and proapoptotic P5C via regulation of PYCR1 activity in response to the intensity of oxidative stress. Positive regulation of mitochondrial complex I is reported as a function of DJ-1 [5,16]. Loss or failure of the function results in inactivation of the complex I and may fall into Parkinson's disease. PYCR1 is also reported to inhibit mitochondria-mediated apoptosis [8]. The additional decrease of mitochondrial membrane potential observed by knockdown of both DJ-1 and PYCR1 suggested that DJ-1 and PYCR1 are involved in different, but cooperative, systems in regulation of mitochondria. The interaction between DJ-1 and PYCR1 is therefore considered as a possible target of pathogenesis/prophylaxis or therapy of Parkinson's disease.

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