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DJ-1 upregulates tyrosine hydroxylase gene expression by activating its transcriptional factor Nurr1 via the ERK1/2 pathway

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ABSTRACT

Loss-of-function DJ-1 mutations have been linked to autosomal recessive early-onset Parikinsonism. However, the putative function of DJ-1 is not completely understood. Previous studies indicate that DJ-1 overexpression results in upregulation of the tyrosine hydroxylase gene. The mechanism by which DJ-1 affects tyrosine hydroxylase expression remains elusive. In the present study, we show that DJ-1 overexpression induces ERK1/2 activation, along with increased tyrosine hydroxylase expression. The L166P DJ-1 mutant, which has been identified as being responsible for familial Parkinsonism, did not have this effect. Moreover, suppression of ERK1/2 phosphorylation by the pharmacological inhibitor U0126 partially abolished the regulating effect of DJ-1 on tyrosine hydroxylase. Nurr1, a transcriptional factor for tyrosine hydroxylase, can be phosphorylated by ERK1/2 and translocate to the nucleus, where it is activated. Thus, we measured nuclear translocation of Nurr1. Confocal microscopy and Western blotting revealed that Nurr1 translocated to the nucleus and was activated by overexpression of wild-type DJ-1, but not of its L166P mutant. Knockdown of Nurr1 gene expression abolished the effect on tyrosine hydroxylase induced by DJ-1. Taken together, these data suggest that DJ-1 upregulates tyrosine hydroxylase expression by activating its transcription factor Nurr1 via the ERK1/2 pathway.

1. Introduction

Parkinson's disease (PD), characterized by resting tremor, rigidity, bradykinesia, gait abnormality and slow movement (Forman et al., 2005), is the most common movement disorder and the second most common neurodegenerative disease. Although predominantly idiopathic, genetic mutations account for ~10% of PD cases (Warner and Schapira, 2003). Studies of genes responsible for familial Parkinsonism/PD are yielding critical insights into mechanisms shared by sporadic and familial disease.

It has been reported that loss-of-function DJ-1 (PARK7) mutations are related to autosomal recessive early-onset Parkinsonism, but little is known about the physiological function of DJ-1 and the mechanisms by which DJ-1 mutations lead to PD. However, research in the last 10 years has revealed several roles for DJ-1. It has a protective role in multiple cell models, including neuronal and non-neuronal cells, against oxidative stress (Aleyasin et al., 2007; Clements et al., 2006; Kim et al., 2005; Meulener et al., 2006; Yang et al., 2005). It was predicted that DJ-1 should act as a chaperone according to its molecular structure (Quigley et al., 2003; Wilson et al., 2004). Subsequent studies revealed that DJ-1 has chaperone activity and inhibits α-synuclein aggregation (Batelli et al., 2008; Deeg et al., 2010; Shendelman et al., 2004; Zhou et al., 2006). Others reported that DJ-1 plays a role in hypoxia (Foti et al., 2010; Vasseur et al., 2009). It has also been shown that DJ-1 may function as a regulator of stroke-induced damage (Aleyasin et al., 2007) and may be a peroxiredoxin-like peroxidase (Andres-Mateos et al., 2007). There is also much evidence indicating that DJ-1 may regulate the expression of some genes. For example, DJ-1 positively regulates the androgen receptor by impairing binding of PIASx alpha to the receptor. (Takahashi et al., 2001). It also regulates gene expression of glutamate–cysteine ligase, the prototypic Nrf2-regulated anti-oxidant enzyme NQO1 and Ret, among others (Clements et al., 2006; Foti et al., 2010; Zhou and Freed, 2005). Our previous study suggested that DJ-1 also upregulates expression of tyrosine hydroxylase (TH), a rate-limiting enzyme in dopamine synthesis, in agreement with a report by Zhong et al. (2006). However, the mechanism involved remains elusive. PD involves progressive degeneration of dopaminergic neurons in the substantia nigra (SN), which leads to decreasing dopamine synthesis. Symptoms appear when 50–70% of dopamine synthesis has been lost. TH is a key enzyme in dopamine synthesis and is a very important factor in both PD pathogenesis and treatment. Elucidation of how DJ-1 regulates TH expression may further our

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understanding of PD pathogenesis and provide useful clues to treatment approaches.

It has been reported that the DJ-1 L166P mutation is responsible for the onset of familial Parkinsonism (Bonifati et al., 2003). L166 is located in the middle of α-helix 8 (α8) and mutation from leucine to proline seems to cause a break in the α-helix. This results in abrogation of dimer formation, which makes the protein unstable and more likely to be degraded (Moore et al., 2003; Blackinton et al., 2005). Interestingly, we found that the L166P mutant did not activate the ERK1/2 pathway as wild-type DJ-1 does (Gu et al., 2009a,b).

In the present study, the wild type DJ-1 gene and its L166P mutant were introduced into dopaminergic MN9D cells and rat brain. Results showed that wild-type DJ-1 overexpression upregulates TH gene expression, together with Nurr1, a transcriptional factor for TH, activation and ERK1/2 phosphorylation. This upregulation can be partly abolished by Nurr1 interference or the MEK1/2 inhibitor U0126. The results indicate that DJ-1 upregulates TH gene expression by activating Nurr1 via the ERK1/2 pathway.

2. Materials and methods

2.1. Constructs

Expression vectors encoding pcDNA3.1, pcDNA3.1-DJ-1 WT and L166P were as previously described (Gu et al., 2009a,b). Human WT-DJ-1 cDNA was obtained by RT-PCR of human brain RNA using the primers 5′-AAG CTT GGG TGC AGG CTT GTA AAC-3′ and 5′-TCT AGA AGT GAT CGT CGC AGT TCG-3′, designed according to the DJ-1 sequence published in GenBank (NM_007262). The cDNA was directionally cloned into the pcDNA3.1 plasmid (Invitrogen). We used an overlapping extension PCR approach to mutate DJ-1. The primers for the L166P mutant were 5′-AGT TTG CGC CTG CAA TTG TT-3′ and 5′-AAC AAT TGC AGG CGC AAA CT-3′. The resulting PCR products were then subcloned into the pcDNA3.1 plasmid. The orientation of the WT and mutated cDNAs in the plasmids was verified by DNA sequencing.

2.2. Cell culture and transfection

MN9D cells were grown in DMEM/F-12 supplemented with 10% newborn calf serum. MN9D cells were transfected with plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were plated at a density of 1×10^5 cells/well in a 24-well plate 1 day before transfection. Samples (1 μg) of pcDNA3.1 or pcDNA3.1-DJ-1/WT or pcDNA3.1-DJ-1/L166P DNA and 2 μL of Lipofectamine 2000 reagent were gently mixed in Opti-MEM (Invitrogen). Cells were incubated at 37 °C in a CO_2 incubator for 24 h after the complex was added. Cells were passaged at a dilution of 1:10 into fresh growth medium and cultured in selective medium (containing 400 μg mL^{-1} zeocin, Invitrogen) to obtain a line of cells stably overexpressing the target gene. The empty pcDNA3.1 vector was used as a negative control.

To obtain protein samples, cells were washed with ice-cold PBS, collected and sonicated in 100 μL of cell lysis solution (50 mmol L^{-1} Tris·Cl, pH 6.8, 15 mmol L^{-1} NaCl, 5 mmol L^{-1} EDTA, 0.5% NP-40, 1 mmol L^{-1} PMSF). The cell lysate was centrifuged at 13,000 × *g* for 30 min at 4 °C and the supernatant was collected. The protein concentration of the cytoplasmic fractions was determined using a BCA protein assay kit (Pierce).

2.3. Nurr1 silencing

Two different oligonucleotide sequence pairs were selected using siRNA software (Invitrogen) for silencing of Nurr1 expression. The siNurr1#1 pair comprised 5′-CCU GUC ACU CUU CUC CUU UTT-3′ and 3′-AAA GGA GAA GAG UGA CAG GTT-5′. The siNurr1#2 pair comprised 5 ′-CCG GCU CUA UGG AGA UCA UTT-3′ and 3′-AUG AUC UCC AUA GAG CCG GTT-5′. One pair of random sequence (scramble) primers was also used (5′-CCA CCU UGC UUG UAC CAA ATT-3′ and 3′-UUU GGU ACA AGC AAG GUG GTT-5′). Dopaminergic MN9D cells (ATCC) carrying the DJ-1 gene were transfected with Lipofectamine 2000 according to the manufacturer's instructions, as described above. Cells were plated in a 6-well plate at a density of 4×10^5 cells/well 1 day before transfection. siRNA (100 pmol) and 5 μL of Lipofectamine 2000 reagent were gently mixed in Opti-MEM (Invitrogen). Cells were incubated at 37 °C in a CO_2 incubator for 6 h after the complex was added and the growth medium was replaced with fresh medium.

2.4. RNA isolation, reverse transcription and qPCR

Total RNA was isolated from human dopaminergic MN9D cells expressing wild type DJ-1 (WT) or its L166P (L166P) mutant using Trizol reagent (Gibco-BRL) according to the recommended protocol. The cells were lysed directly using 1 mL of Trizol reagent in a 35-mm-diameter dish. Cell lysates were transferred into tubes and 0.2 mL of chloroform was added to extract total RNA. The samples were centrifuged at 12,000 × *g* for 15 min at 4 °C, which yielded a lower red, phenol–chloroform phase, an interphase, and a colorless upper aqueous phase containing the RNA. The aqueous phase was transferred to a fresh tube and the RNA was precipitated by mixing with isopropyl alcohol. The precipitate formed a gel-like pellet on the side and bottom of the tube after centrifugation at 12,000 × *g* for 10 min at 4 °C. The supernatant was removed and the RNA pellet was washed once with 75% ethanol and dried for 10 min. The RNA was dissolved in RNase-free water. After DNase treatment, RNA samples (5 μg) were reverse-transcribed in the presence of 1 μL of dNTPs (10 mmol L^{-1}, Invitrogen) and 0.5 μg of oligo(dT) with 50 U of Superscript™ RNase reverse transcriptase (Invitrogen) for 50 min at 42 °C. The following primers were used: β-actin, forward 5′-ACC ACC ATG TAC CCA GGC ATT-3′ and reverse 5′-CCA CAC AGA GTA CTT GCG CTC A-3′; DJ-1, forward 5′-GAG GTG GCG GCT CAA GTG-3′ and reverse 5′-CGC AGT TCG CTG CTC TAG TCT-3′; Nurr1, forward and reverse as above; and TH, forward 5′-CGA GCT GCT GGG ACA CGT A-3′ and reverse 5′-CTG GGA GAA CTG GGC AAA TG-3′. PCR was performed using 7300 and 7500 real-time PCR systems (Applied Biosystems) and the default thermal cycle conditions (2 min at 50 °C plus 10 min at 95 °C for the hot start, and then 40 cycles of 15 s at 95 °C plus 1 min at 60 °C for amplification).

2.5. Nuclear and cytoplasmic protein fractionation from MN9D cells

Cytoplasmic and nuclear extracts were prepared from different groups of MN9D cells (Con, WT and L166P groups). When cells reached 80% confluence, the culture medium was removed and the cells were rinsed with PBS. Cells were collected by centrifugation for 5 min at 800 × *g* and were washed twice with PBS after trypsinization. Then 100 μL of Cytosol Extraction Buffer A (Apply Gen) was added to the cells. After 10 min of incubation, 5 μL of Cytosol Extraction Buffer B (Apply Gen) was added and cells were incubated on ice for 1 min and then centrifuged for 5 min at 1000 × *g* at 4 °C. The pellet contained crude nuclei. The supernatant was transferred to a new tube and further centrifuged for 10 min at 12,000 × *g* at 4 °C. The resulting supernatant was the cytosol extract. The nuclear pellet was washed once with 100 μL of Cytosol Extraction Buffer A and then 20 μL of Nuclear Extraction Buffer was added. The complex was incubated on ice for 30 min with vortexing every 10 s for 5 min and then centrifuged for 10 min at 12,000 × *g* at 4 °C.

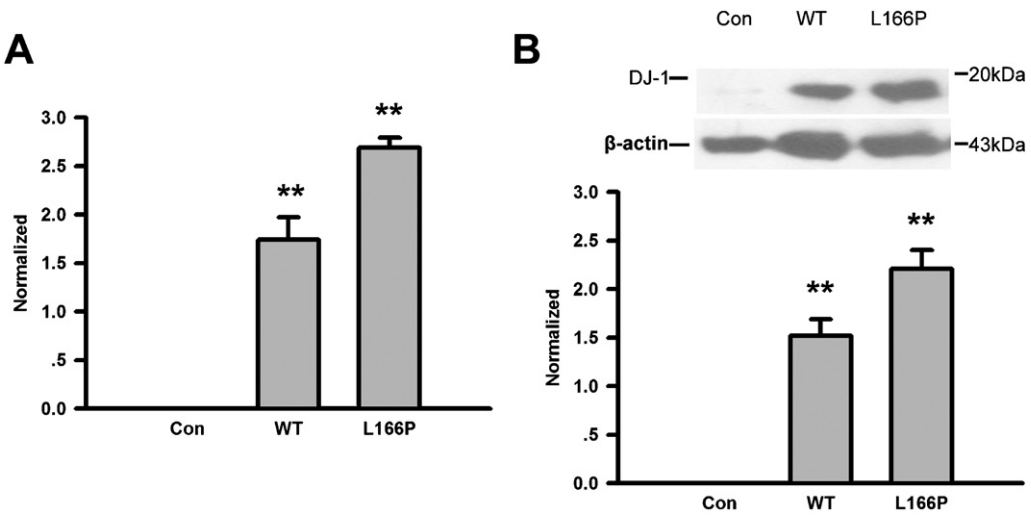

Fig. 1. The exogenous human DJ-1 is stably overexpressed in MN9D cells. (A) Real-time quantitative RT-PCR of DJ-1. A pair of specific primer was designed according to human DJ-1 gene to detect the exogenous DJ-1 mRNA level in MN9D cells. The human DJ-1 mRNA level was normalized to TH mRNA in dopaminergic MN9D cells. (B) Western blot analysis of exogenous human DJ-1 protein levels. Cell lysates were extracted from stable Con, WT and L166P cell clones. For quantification, protein levels were normalized to TH protein levels of MN9D cells. Experiments were performed in triplicate. The results show that wild-type DJ-1 and its L166P mutant were expressed at high levels in MN9D cells respectively, which confirms that stable cell lines overexpressing wild-type DJ-1 or its L166P mutant were obtained. $^{**}p < 0.01$.

The supernatant fraction contained proteins extracted from nuclei and was transferred to a clean tube and stored at −40 °C.

2.6. Western blot analysis

Equal amounts of protein (100 μg) were separated by 10% SDS-PAGE and then transferred onto nitrocellulose blotting membranes (Pall Co.). The membranes were blocked in 5% non-fat milk for 1 h at room temperature and incubated overnight with the primary antibody. The following antibodies were used: anti-DJ-1 (1:5000, Sigma), anti-Nurr1 (1:1000, Santa Cruz), anti-TH (1:10,000, Sigma), anti-DDC (1:1000, Abcam), anti-VMAT-2 (1:1000, Santa Cruz), anti-ERK1/2 (1:1000, CST) and anti-β-actin (1:1000, Sigma). For development, anti-mouse HRP and anti-rabbit HRP (1:10,000, Sigma) were used in combination with ECL reagent (GE Healthcare).

2.7. Statistical analysis

All experiments were repeated in triplicate at least. Data represent the mean ± SEM. Each group was compared individually with the reference control group using one-way ANOVA with a Tukey post hoc test (SPSS 11.5). The relationship between TH, Nurr1 and pERK1/2 protein levels is analyzed by using multivariate correlations method. All measurements were performed separately by two skilled researchers.

3. Results

3.1. Generation of stable cell lines expressing wild-type DJ-1 or its L166P mutant

To obtain cell lines stably overexpressing wild-type DJ-1 or its L166P mutant, we transfected the target gene into dopaminergic MN9D cells. After transfection, cells were cultured in selective medium containing zeocin (400 μg mL^{-1}). Cell clones overexpressing wild type DJ-1 (WT) or its L166P mutant (L166P) or an empty vector (control, Con) were obtained after 2 months. The three stable clones were then analyzed for DJ-1 expression levels. As shown in Fig. 1, WT and L166P clones showed a significant increase in DJ-1 mRNA and protein levels. Thus, we successfully generated a dopaminergic cell model for studying the effect of DJ-1 and its L166P mutant in vitro.

3.2. Overexpression of DJ-1 upregulates TH gene expression in vitro

TH mRNA and protein levels were measured in dopaminergic MN9D cells carrying an empty vector, wild-type DJ-1 or its L166P mutant by quantitative real-time RT-PCR (qPCR) and Western blotting. qPCR analysis showed that TH mRNA levels in WT clones increased approximately 20-fold compared to control cells. By contrast, TH mRNA expression did not increase so much remarkably in L166P cells than in WT (Fig. 2). Western blot analysis also showed that TH was upregulated by overexpression of wild-type DJ-1, but not of its L166P mutant (Fig. 2).

3.3. DJ-1 upregulates TH by activating its transcriptional factor Nurr1

It has been reported that Nurr1 is a transcriptional factor for TH and that TH can be upregulated when Nurr1 is activated (Hermanson et al., 2003; Jacobsen et al., 2008; Zhang et al., 2007). It is well known that transcriptional factors are translocated to the nucleus when activated, so we extracted cytosolic and nuclear proteins separately to investigate if Nurr1 is translocated to the nucleus and activated. The results showed that that Nurr1 nuclear translocation occurred in the WT group, but not in the L166P and Con groups (Fig. 3). This nuclear translocation was verified by immunostaining and confocal microscopy (Fig. 3).

To prove that the mechanism of TH upregulation strictly depends on Nurr1 activation, we performed transient knockdown experiments for Nurr1. Dopaminergic MN9D cells were transfected with siNurr1 #1, siNurr1 #2, scramble (Scramble control) or water (mock control) for 6 h. As shown in Fig. 4, both siNurr1 #1 and siNurr1 #2 successfully knocked down Nurr1 expression, with siNurr1 #1 being more efficient. Thus, siNurr1 #1 was used in subsequent experiments. Moreover, TH protein levels were consistently decreased by Nurr1 silencing.

We then performed similar experiments in the WT group carrying the wild-type DJ-1 gene. We transiently transfected these DJ-1-overexpressing cells with siNurr1 #1. TH and Nurr1 protein

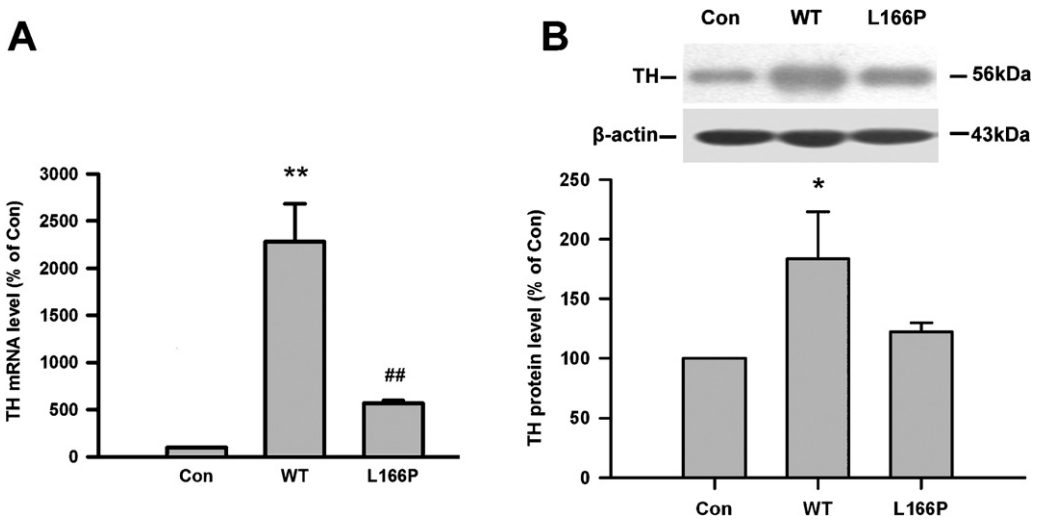

Fig. 2. Overexpression of DJ-1 upregulates TH gene expression in vitro. (A) Real-time RT-PCR analysis of TH mRNA in Con, WT and L166P. The results showed that TH mRNA levels were significantly higher in WT, but not in L166P compared to Con. (B) Western blot analysis of TH protein levels in MN9D cells. Cell lysates were prepared from Con, WT and L166P. For quantification, protein levels were normalized to β-actin levels and compared to Con. The results show that TH protein levels are upregulated by overexpression of wild-type DJ-1, but not of its L166P mutant. Experiments were performed in triplicate. Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with Con; $\#\#p < 0.01$ compared with WT.

levels were then analyzed by Western blotting. As shown in Fig. 4, the reduction in Nurr1 was strictly correlated with a decrease in TH expression. Therefore, TH protein levels in dopaminergic MN9D cells were sensitive to DJ-1 overexpression in a Nurr1-dependent manner.

To further ascertain the specificity of Nurr1-mediated TH upregulation, we transfected cells carrying the L166P DJ-1 mutant gene with pBK-RSV-Nurr1 or with the pBK-RSV empty vector as a negative control. TH and Nurr1 protein levels were then monitored by Western blotting. The results show that TH was upregulated by Nurr1 overexpression in the L166P group (Fig. 4).

3.4. DJ-1 upregulates TH via the ERK1/2 pathway

It has been reported that MAPK increases Nurr1 transcriptional activity and that Nurr1 is a substrate for ERK1/2 (Zhang et al., 2007). We thus postulated that ERK1/2 might play a role in DJ-1-mediated Nurr1 activation. We first measured phospho-ERK1/2 levels in WT, L166P and Con groups. ERK1/2 phosphorylation was greater in WT compared to the L166P and Con groups (Fig. 5), which suggests that DJ-1 activates Nurr1 via ERK1/2 phosphorylation.

To further prove the above hypothesis, we blocked the ERK1/2 pathway using an MEK1/2 inhibitor, U0126, and monitored TH protein expression and Nurr1 translocation. Western blotting revealed that the TH upregulation mediated by DJ-1 can be downregulated by U0126 (Fig. 6), which indicates that ERK1/2 is involved, at least in part, in the regulation process. Furthermore, Nurr1 nuclear translocation and transcriptional activation resulting from DJ-1 overexpression were reversed.

The results of the bivariate correlation analyses between pERK1/2, nuclear Nurr1 and TH are presented in Table 1. It is notable that the most frequent significant correlations were found between

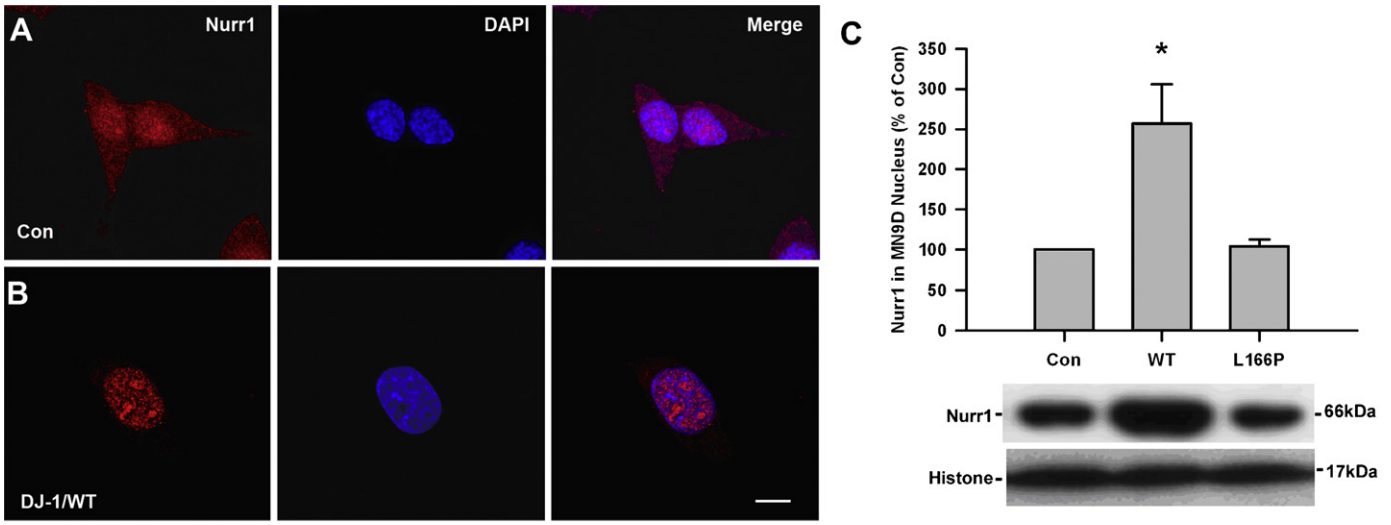

Fig. 3. Nuclear translocation of Nurr1 is induced by DJ-1 overexpression. (A and B) Nurr1 translocalized to the nucleus when DJ-1 was overexpressed in MN9D cells. MN9D cells transfected with pcDNA3.1 (Con, A) and pcDNA3.1-DJ-1 (DJ-1/WT, B) were immunostained for Nurr1 (1:200, red) and stained for DAPI (nuclear stain, blue) and detected by confocal microscopy. Scale bar, 10 μM. (C) Nuclear proteins were extracted from Con, WT and L166P cell clones. The results show that more Nurr1 nuclear translocalization occurred in the WT group, which confirms that Nurr1 was activated. Experiments were performed in triplicate. Data are presented as mean ± SEM; $^{*}p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

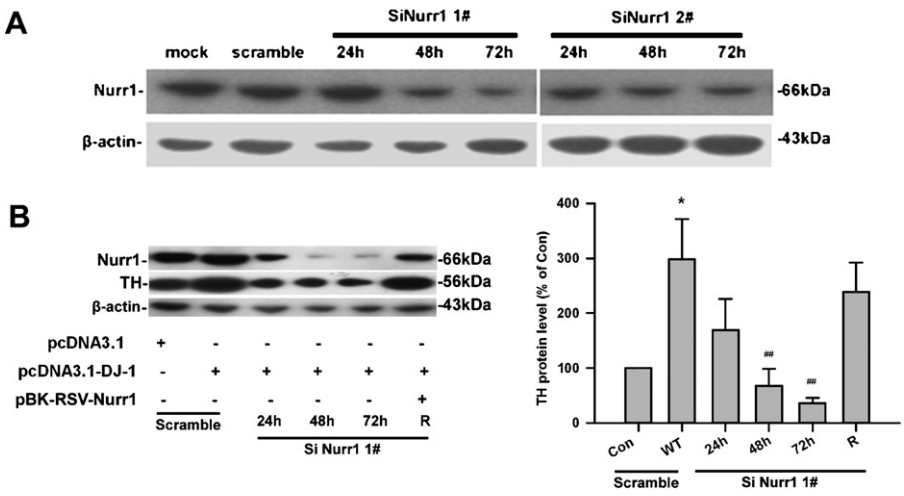

Fig. 4. TH protein levels are sensitive to overexpression of wild-type DJ-1 in a Nurr1-dependent manner in dopaminergic MN9D cells. (A) Representative Western blot analysis of Nurr1 protein levels. Cell lysates were extracted from MN9D cells transfected with water instead of siRNA sequence (mock), MN9D cells with random sequence (scramble), MN9D cells with SiNurr1 1# (SiNurr1 1#)and MN9D cells with SiNurr1 2# (SiNurr1 2#). The results show that both SiNurr1 1# and 2# effectively knocked down Nurr1 expression, although SiNurr1 1# was more efficient. (B) Overexpression of DJ-1 resulted in TH upregulation. However, it was downregulated to a lower level when Nurr1 was knocked down. This downregulation effect can be recovered when Nurr1 is overexpressed. Data are presented as mean ± SEM; $^{*}p < 0.05$ compared with Con/Scramble. $^{\#\#}p < 0.01$ compared with WT/Scramble.

pERK1/2 and nuclear Nurr1. It is also remarkable that statistical analyses revealed significant correlations between nuclear Nurr1 levels and TH levels. Nonetheless, there is also a significant correlations between pERK1/2 levels and TH levels.

In summary, DJ-1 overexpression upregulates TH expression by activating Nurr1 transcriptional activity via the ERK1/2 pathway.

4. Discussion

Studies have shown that DJ-1 plays an important role in anti-oxidative stress. Tolerance to neurotoxins such as paraquat and rotenone is partly mediated by DJ-1 self-oxidation, which leads to an acidic p*I* shift (Gorner et al., 2007; Kahle et al., 2009; Waak et al., 2009). Studies have shown that DJ-1 self-oxidation is also observed in the brain of ageing individuals (Im et al., 2010; Saeed et al., 2009). Moreover, it has been reported that DJ-1 can rescue PINK1 deficiency (Kitada et al., 2009). Taken together, these data indicate that DJ-1 may be related to several key risk factors in PD, including genetic, oxidative stress and environmental factors. Although DJ-1 is implicated in only 1–2% of early-onset PD cases (Bonifati et al., 2003), numerous studies have focused on this protein. However, the exact physiology of DJ-1 functions and the pathology associated with DJ-1 mutations remain unclear.

Our study shows that wild-type DJ-1, but not its L166P mutant, regulates TH expression, which is consistent with previous studies (Ishikawa et al., 2010; Zhong et al., 2006). TH is an enzyme belonging to the family of aromatic amino acid hydroxylases and catalyzes the first reaction in the catecholamine synthesis pathway. In the presence of tetrahydrobiopterin and oxygen co-substrates, TH converts tyrosine to DOPA (Nagatsu et al., 1964). By influencing the efficiency of DOPA synthesis, TH determines the proper functioning of the entire dopaminergic system (Levitt et al., 1965). TH also determines the course of many physiologic processes, including voluntary movement, and is involved in emotional conditioning (Lenartowski and Goc, 2011). Hence, regulation of TH expression may be a newly identified function for DJ-1 that is important for PD occurrence and progression. The mechanism for this regulation is not exactly understood. Zhong et al. (2006) reported that it involves inhibition of the sumoylation of pyrimidine tract-binding protein-associated splicing factor. Here, we report a novel mechanism whereby DJ-1 upregulates TH protein expression by activating its transcriptional factor Nurr1.

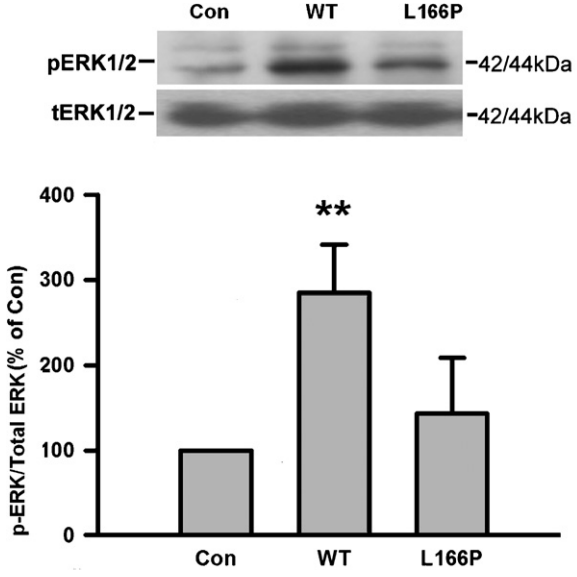

Fig. 5. The ERK1/2 pathway is activated by DJ-1 overexpression in vitro. Cell lysates were extracted from stable Con, WT and L166P cell clones. The results show that phospho-ERK1/2 levels greatly increased in the WT group, but not in the L166P group compared to Con. For quantification, pERK1/2 levels were normalized to tERK1/2 levels and compared to the Con groups. Experiments were performed in triplicate. Data are presented as mean ± SEM. $^{**}p < 0.01$ compared with Con.

Table 1
Relationships (correlation coefficients and significance of the correlation) between pERK1/2, nuclear Nurr1 and TH protein levels ($n = 15$).

		pERK	nNurrl	TH
pERK	Pearson correlation coefficient	1	.759**	.865**
	Significance of the correlation		.001	.000
nNurr1	Pearson correlation coefficient	.759**	1	.762**
	Significance of the correlation	.001		.001
TH	Pearson correlation coefficient	.865**	.762**	1
	Significance of the correlation	.000	.001	

** $p < 0.01$.

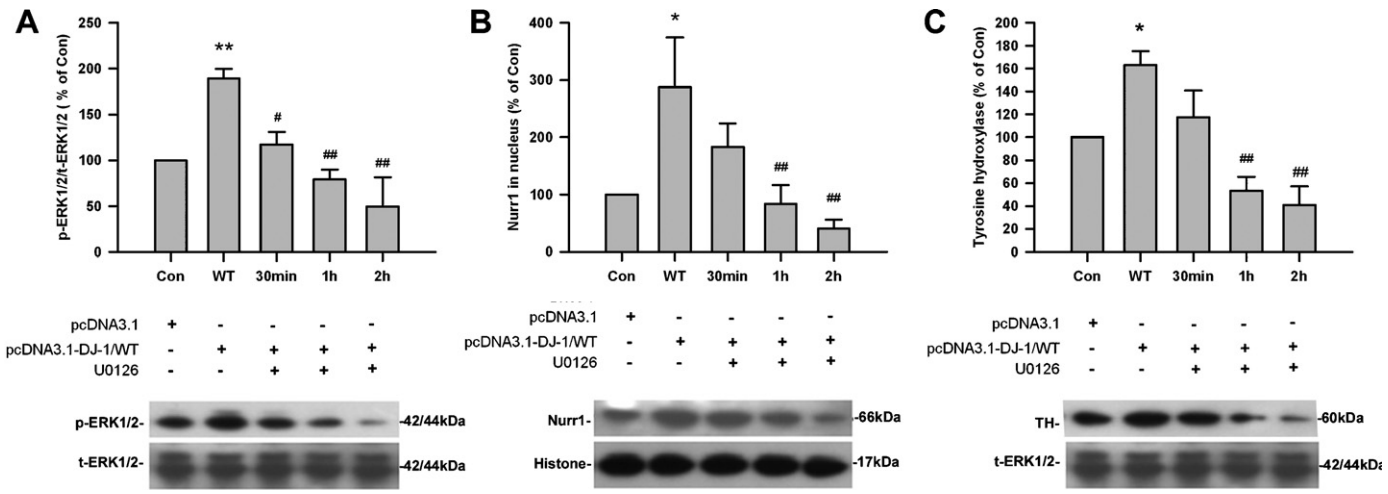

Fig. 6. TH upregulation mediated by DJ-1 is downregulated by the MEK1/2 inhibitor U0126. MN9D cells were treated with the MEK1/2 inhibitor U0126 (10 μM) for 30 min, 1 h and 2 h. Whole-cell lysates were analyzed by Western blotting to detect TH, phospho-ERK1/2 and total ERK1/2 levels and nuclear proteins were analyzed to detect Nurr1 translocation as indicated. Data are presented as mean ± SEM. $^{*}p < 0.05$ compared with Con; $\#p < 0.05$ compared with WT; $\#\#p < 0.01$ compared with WT.

As Nurr1 is a transcriptional factor for TH and TH is upregulated when Nurr1 is translocalized to the nucleus and activated (Hermanson et al., 2003; Jacobsen et al., 2008), we measured its nuclear translocation. We found that Nurr1 was activated by overexpression of wild-type DJ-1, but not of its L166P mutant. This is the first report of DJ-1 activation of Nurr1. Furthermore, TH upregulation by DJ-1 can be abolished when Nurr1 is silenced. These data suggest that DJ-1 upregulates TH expression by activating its transcription factor, Nurr1.

Our previous study indicated that DJ-1 overexpression results in activation of the ERK1/2 pathway (Gu et al., 2009a,b). Moreover, studies have shown that two regions in Nurr1, the N-terminal (AF1) and C-terminal (AF2) regions, are important for its transcriptional activation (Nordzell et al., 2004). AF1 in Nurr1 is important for regulating transcription in a mitogen-activated protein kinase (MAPK)-dependent manner (Nordzell et al., 2004). Moreover, it has been reported that Nurr1 can be phosphorylated by ERK1/2 and translocalized to the nucleus and thus upregulates TH expression (Jacobsen et al., 2008). To elucidate the mechanism by which DJ-1 activates Nurr1, we measured changes in the ERK1/2 pathway by Western blotting. Interestingly, the ERK1/2 pathway was activated when DJ-1 was overexpressed. Addition of U0126, an MEK1/2 inhibitor, reversed Nurr1 nuclear translocation and TH upregulation. Multivariate correlations analysis showed that there is a significant correlations between TH, nuclear Nurr1 and pERK1/2 protein levels. Taken together, the results indicate that DJ-1 can upregulate TH expression by activating Nurr1 via the ERK1/2 pathway.

U0126 is a fast and potent inhibitor of MEK1/2, which is the upstream kinase for ERK1/2. PhosphoERK1/2 decreased to a very low level when cells were treated with 10 μM U0126 for only 4 min (McNicol and Jackson, 2003). Inhibition of the ERK1/2 pathway influences the expression of some genes, such as Cyp1a1, Serpinb2 (Kennedy et al., 2006), TNF-α, IL-1α, IL-1β, MIP-1α (Wang et al., 2004) and endothelin ETB receptor (Sandhu et al., 2010). Some genes, such as Cyp1a1, Serpinb2, TNF-α, IL-1α, IL-1β and MIP-1α, show a rapid response to ERK1/2 pathway inhibition. Levels of proteins encoded by these genes changed significantly on incubation with U0126 for only 2–4 h. Other genes, such as endothelin ETB receptor and glial fibrillary acidic protein (Lind et al., 2006), respond to the ERK1/2 pathway over a longer time. U0126 treatment for 24–48 h is required for a change in protein levels. In the present study, TH expression decreased to a relatively low level after 1–2 h of incubation with U0126, which indicates that TH expression may play a role as a rapid response to the ERK1/2 pathway.

Overexpression of the L166P mutant had no effect on TH expression, Nurr1 translocation or ERK1/2phosphorylation. It has been reported that the DJ-1 L166P mutation is responsible for the onset of familial PD (Bonifati et al., 2003). L166 is located at the middle of α-helix 8 and mutation from leucine to proline seems to cause a break in the α-helix. This results in abrogation of dimer formation, making the protein unstable and more likely to be degraded (Deeg et al., 2010). Dimer formation is thought to be necessary for DJ-1 functions. Partial degradation and loss of dimer formation may be a contributory factor to the loss of function for the L166P mutant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocel.2011.09.007.

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