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Astrocyte-specific DJ-1 overexpression protects against rotenone-induced neurotoxicity in a rat model of Parkinson's disease

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ABSTRACT

DJ-1 is a redox-sensitive protein with several putative functions important in mitochondrial physiology, protein transcription, proteasome regulation, and chaperone activity. High levels of DJ-1 immunoreactivity are reported in astrocytes surrounding pathology associated with idiopathic Parkinson's disease, possibly reflecting the glial response to oxidative damage. Previous studies showed that astrocytic over-expression of DJ-1 *in vitro* prevented oxidative stress and mitochondrial dysfunction in primary neurons. Based on these observations, we developed a pseudotyped lentiviral gene transfer vector with specific tropism for CNS astrocytes *in vivo* to overexpress human DJ-1 protein in astroglial cells. Following vector delivery to the substantia nigra and striatum of adult Lewis rats, the DJ-1 transgene was expressed robustly and specifically within astrocytes. There was no observable transgene expression in neurons or other glial cell types. Three weeks after vector infusion, animals were exposed to rotenone to induce Parkinson's disease-like pathology, including loss of dopaminergic neurons, accumulation of endogenous α-synuclein, and neuroinflammation. Animals over-expressing hDJ-1 in astrocytes were protected from rotenone-induced neurodegeneration, and displayed a marked reduction in neuronal oxidative stress and microglial activation. In addition, α-synuclein accumulation and phosphorylation were decreased within substantia nigra dopaminergic neurons in DJ-1–transduced animals, and expression of LAMP-2A, a marker of chaperone mediated autophagy, was increased. Together, these data indicate that astrocyte-specific over-expression of hDJ-1 protects neighboring neurons against multiple pathologic features of Parkinson's disease and provides the first direct evidence *in vivo* of a cell non-autonomous neuroprotective function of astroglial DJ-1.

1. Introduction

DJ-1 is a pleiotropic protein containing a ThiJ/PfpI domain characteristic of a protein chaperone, and is expressed ubiquitously in all human cell types (Nagakubo et al., 1997; Lee et al., 2003; Tao and Tong, 2003; Wilson et al., 2005). Autosomal recessive mutations in the PARK7 gene encoding DJ-1 are linked to a rare, familial type of early-onset parkinsonism (Bonifati et al., 2002, 2003; Tao and Tong, 2003). However, even in cases of idiopathic Parkinson's disease (PD) with no apparent PARK7 mutations, postmortem analysis reveals that high levels of oxidized DJ-1 accumulate in neurons and astrocytes of the ventral midbrain (Bandopadhyay et al., 2004; Choi et al., 2006).

Multiple lines of evidence suggest that, within neurons, DJ-1 is involved in maintaining mitochondrial function (Miller et al., 2003; Canet-Avilés et al., 2004; Blackinton et al., 2005; Hayashi et al., 2009; Junn et al., 2009; Larsen et al., 2011; Thomas et al., 2011), protein transcription and processing (Takahashi et al., 2001; Shinbo et al., 2005; Xu et al., 2005; Zhong et al., 2006; Lu et al., 2016), neurotransmitter reuptake (Luk et al., 2015; Piston et al., 2017), proteasome activity (Saito et al., 2016; Moscovitz et al., 2017), and signal transduction (Aleyasin et al., 2010; Zhang et al., 2017; reviewed in Oh and Mouradian, 2017), abnormalities of which are implicated in the pathogenesis of PD. A highly conserved (Bandyopadhyay and Cookson, 2004; Bai et al., 2006), and readily oxidized cysteine (Cys)106 residue suggests that DJ-1 functions in part as a redox sensor (Canet-Avilés et al., 2004; Taira et al., 2004; Kinumi et al., 2004; Ooe et al., 2006; Blackinton et al., 2009b), and is induced in substantia nigra (SN) dopamine neurons that experience a high degree of oxidative burden (Lin and Beal, 2006; Wang and Michaelis, 2010; Winklhofer and Haass, 2010; Surmeier et al., 2017). Additionally, DJ-1 expression has been inversely correlated with α-synuclein toxicity and accumulation, both through mechanisms of direct protein interaction (Shendelman et al.,

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2004; Meulener et al., 2005; Zhou et al., 2006; Zondler et al., 2017), as well as the transcriptional upregulation of chaperone proteins (*i.e.* HSP70; Batelli et al., 2008; Xu et al., 2017).

DJ-1 has also been shown to be important in maintaining astrocytic mitochondrial function (Ashley et al., 2009; Larsen et al., 2011), and impairment of DJ-1 in astrocytes deleteriously affects primary neurons following an oxidative insult (Mullett and Hinkle, 2011, 2009; Mullett et al., 2012). Conversely, *in vitro* overexpression of DJ-1 in astrocytes is protective against rotenone-induced mitochondrial dysfunction and reactive oxygen species (ROS) production in co-cultured neurons (Mullett et al., 2012). Collectively, these data suggest that astrocytic DJ-1 plays a cell non-autonomous role in the protection of neurons from the consequences of mitochondrial dysfunction.

In a previous study, we discovered that replication-defective HIV1-based vectors pseudotyped with the envelope glycoprotein from Moloney Murine Leukemia Virus (MuLV) showed specific tropism for astrocytes within the adult rat brain (Cannon et al., 2011). Here, we engineered a MuLV-pseudotyped HIV1-based vector to overexpress human DJ-1 (hDJ-1) protein selectively within astrocytes, but not in neurons or other glial cells (microglia or oligodendrocytes). This new vector allowed us to test whether modulation of astrocytic DJ-1 expression could attenuate neurodegeneration *in vivo*. Our data show unequivocal evidence that astroglial DJ-1 over-expression is neuroprotective in the rat rotenone model of PD, thereby providing the first direct evidence of a cell non-autonomous protective function of astrocyte DJ-1 *in vivo*.

2. Materials and methods

2.1. Chemical reagents and supplies

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Antibodies are listed in Table 1.

2.2. Lentiviral vectors

Total RNA (RNAqueous, Invitrogen, Carlsbad, CA) was isolated from human NT2 cells and reverse transcribed using oligo-dT primers (SuperScript III, Invitrogen). cDNA encoding human DJ-1 was amplified by PCR with Platinum *Pfx* polymerase (Invitrogen) using primers hDJ-1F-EcoR1 (5′-CGAATTCGGTGCAGGCTTGTAAACATA-3′) and hDJ-1R-*Kpn*I (5′-CGGTACCTGAGAATGGATTCCTAACGG-3′). The PCR product was digested with *Eco*RI and *Kpn*I and inserted into the *Eco*RI/*Kpn*I sites of pBlueScript to make pBS-DJ-1, which was sequenced to verify that no mutations were introduced during amplification. The human DJ-1 ORF was released from pBS-DJ-1 and inserted into the *Eco*RI/*Sma*I sites of pIRES2-EGFP (Clontech, Mountain View, CA) to yield pDJ-1-IRES2-EGFP. This was digested with *Xba*I, the 5′ overhang filled in using Klenow fragment and then digested with *Eco*RI to liberate a fragment containing hDJ-1-IRES-EGFP. The HIV-1-based lentiviral gene transfer plasmid pHR′ (Naldini et al., 1996) was digested with *Bam*HI/*Xho*I to release the LacZ insert and re-ligated, generating pHR′-ΔLacZ. pHR′-ΔLacZ was digested with *Kpn*I, the 3′ overhang removed using Klenow fragment, and then digested with *Eco*RI, so that the hDJ-1-IRES-EGFP could be ligated into the resulting blunt and *Eco*RI ends to yield pHR′-hDJ-1-IRES2-EGFP, with the DJ-1 ORF immediately 3′ of the CMV promoter. The control vector pHR′-IRES2-EGFP was generated similarly except for the absence of the DJ-1 gene. Production of transduction-competent lentiviral particles from these plasmids was confirmed *in vitro* by co-transfection of 293T cells with packaging and VSV-G envelope plasmids. Expression of DJ-1 was verified by Western blot analysis of cells transduced by the resulting viral particles, expression of GFP was confirmed by epifluorescence microscopy of transduced cells, and biological activity of over-expressed DJ-1 was confirmed by protection of transduced PC6-3 cells from H_2O_2 exposure (Supplemental Fig. 1). High titer stocks of MuLV-pseudotyped lentiviral vector particles of sufficient purity for *in vivo* transduction were generated by 3-plasmid transfection at the University of Pennsylvania Gene Therapy Vector Core. The presence of replication-competent lentivirus in the resulting viral stocks was excluded by monitoring p24 antigen levels in the culture medium for 30 days after transduction of 293T cells. DJ-1 and control vector stocks were diluted in PBS to the same titer (1.78×10^9 GC/ml) prior to experiments.

Table 1
Antibodies for immunohistochemistry.

Antibody	Catalog	Company
Tyrosine hydroxylase	AB1542	EMD Millipore (Burlington, MA)
Anti-nitrotyrosine	06-284	
Glial fibrillary acidic protein (GFAP)	mAb #3670	Cell Signaling Technology (Danvers, MA)
Human DJ-1/PARK7	Ab18257	Abcam (Cambridge, MA)
Ser129-α-synuclein	Ab51253	
Iba1	019-19741	Wako Chemicals USA (Irvine, CA)
NDUFS3/OxPhos	459130	Thermo Fisher (Waltham, MA)
LAMP-2A	51-2200	
TOM20	SC-11415	Santa Cruz Biotechnology (Santa Cruz, CA)
CD-68 (ED1)	MCA341	BioRad (Hercules, CA)
α-Synuclein	610787	BD Biosciences (San Jose, CA)

2.3. Animals and stereotaxic surgery

Adult, middle-age (7–9 months), male Lewis rats (Charles River Laboratories, Wilmington, MA) were used for all experiments. Conventional diet and water were given *ad libitum*. Animals were maintained under standard temperature-controlled conditions with 12:12 hour light-dark cycle. Rats were randomly assigned to control and treatment groups and blinded throughout all study analyses. All experiments involving animal treatment and euthanasia were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Rodent stereotaxic surgery was performed under deep isoflurane anesthesia. Each animal was infused unilaterally with 2 µl of LV-hDJ-1/MuLV or LV-GFP/MuLV following standard Bregma coordinates for the substantia nigra (Bregma −5.8 mm A/P, −2.2 mm M/L, −8.5 mm V). Striatal infusion of 4 µl of either vector was performed at two injection depths (2 µl per site) to maximize vector expression throughout the dorsolateral striatum (Bregma +1.2 mm A/P, −3.4 mm M/L, −5; −7 mm V).

2.4. Rotenone administration and motor behavior

One week after virus infusion, rats started a 2-week behavioral training period, after which they were given single daily intraperitoneal (IP) injections of 2.8 mg/kg of rotenone suspended in 2% DMSO, 98% Miglyol 812 N as described (Cannon et al., 2009). Motor deficits were assessed daily using the postural instability test (PIT; Woodlee et al., 2008) until each rat reached its motor behavioral endpoint, defined as the rat's inability to successfully complete the PIT or loss of 25% body mass (Supplemental Fig. 2). Behavioral tests were carried out by investigators blinded to treatment group throughout the study. Animals were euthanized using 0.3 mg/kg pentobarbital, followed by transcardial perfusion and 4% paraformaldehyde perfusion fixation.

2.5. Striatal terminal intensity

Brain sections (35 µm) through striatum were stained for tyrosine hydroxylase (TH) using immunofluorescence. Striatal tissue sections were analyzed using near-infrared imaging for density of dopamine neuron terminals (LiCor), and analyzed using Odyssey software (V3.0).

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