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DJ-1 Changes in G93A-SOD1 Transgenic Mice: Implications for Oxidative Stress in ALS

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Abstract Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, neurodegenerative disorder. The causes of ALS are still obscure. Accumulating evidence supports the hypothesis that oxidative stress and mitochondrial dysfunction can be implicated in ALS pathogenesis. DJ-1 plays an important role in the oxidative stress response. The aim of this study was to discover whether there are changes in DJ-1 expression or in DJ-1-oxidized isoforms in an animal model of ALS. We used mutant $SOD1^{G93A}$ transgenic mice, a commonly used animal model for ALS. Upregulation of DJ-1 mRNA and protein levels were identified in the brains and spinal cords of $SOD1^{G93A}$ transgenic mice as compared to wild-type controls, evident from an early disease stage. Furthermore, an increase in DJ-1 acidic isoforms was detected, implying that there are more oxidized forms of DJ-1 in the CNS of $SOD1^{G93A}$ mice. This is the first report of possible involvement of DJ-1 in ALS. Since DJ-1 has a protective role against oxidative stress, it may suggest a possible therapeutic target in ALS.

Keywords Amyotrophic lateral sclerosis (ALS) · DJ-1 · Cu/Zn superoxide dismutase (SOD1) · Oxidative stress

Introduction

Amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease and motor neuron disease) is a progressive, lethal, neurodegenerative disorder. The hallmark of the disease is the selective death of motor neurons in the brain and spinal cord, leading to paralysis of voluntary muscles. Respiratory failure is the cause of death and typically occurs within 5 years. ALS has a prevalence of 1–2 per 100,000 people and is the most common motor neuron disease affecting adults. Most ALS patients suffer from a sporadic disease. However, in the recent years, accumulating data on genetic causes of ALS is emerging. These genetic causes helped develop animal models for the study of ALS, enabling to elucidate the pathogenetic mechanisms operating in the basis of the disease. Five to ten percent of all human ALS cases have a familial cause (fALS); of which, about 20% are caused by a mutation in the gene-encoding Cu/Zn superoxide dismutase (SOD1). The most commonly used rodent model of ALS exploits the consequence of a glycine to alanine mutation at position 93 of human SOD1 ($SOD1^{G93A}$ mice; Gurney et al. 1994). These mice replicate much of the human ALS phenotype. These mice lose motor neurons and develop progressive paralysis and die at about 4 months of age (Gurney et al. 1994).

Oxidative stress, mitochondrial dysfunction, excitotoxicity, neuroinflammation, protein misfolding, neurotrophic factors, and altered axonal transport were implicated in the etiology and possible therapeutic targets in ALS. In humans, as well as animal models, it has been shown that oxidative stress plays a central role in the progression of motor neuron loss, possibly in concert with a chronically enhanced excitotoxic profile and neuroinflammation (Andrus et al. 1998; Agar and Durham 2003; Carri et al. 2003; Hensley et al. 2006; Kraft et al. 2007; Harraz et al. 2008).

DJ-1 encodes a small 189 amino acid protein that is ubiquitously expressed and highly conserved throughout diverse species (Nagakubo et al. 1997; Bandopadhyay et al. 2004). DJ-1 is widely distributed and is highly expressed in

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the central nervous system (CNS) and is not confined to a single anatomical or functional system (Bader et al. 2005). DJ-1 mutations are known to cause early onset autosomal recessive Parkinson's disease (Bonifati et al. 2003). Recently, three brothers with DJ-1 mutations were reported to be suffering from symptoms of both Parkinson's disease and motor neuron disease (Annesi et al. 2005). Accumulating evidence suggests that DJ-1 responds to oxidative stress (Mitsumoto et al. 2001; Canet-Aviles et al. 2004). DJ-1 has several isoforms with different isoelectric points (pI). Upon exposure to oxidative stress, there is a shift of DJ-1 isoforms towards more acidic isoforms. This pI shift is caused by the oxidation of cysteine and methionine residues in DJ-1 (Mitsumoto et al. 2001; Canet-Aviles et al. 2004; Choi et al. 2006). This shift was also verified in human patients—postmortem brain samples taken from PD patients found that the acidic isoforms of DJ-1 were more abundant in PD brains as compared to controls (Bandopadhyay et al. 2004; Choi et al. 2006).

The aim of this study was to discover whether there are changes in DJ-1 expression or in DJ-1-oxidized isoforms in an animal model of ALS, $SOD1^{G93A}$ transgenic mice, and whether there is a correlation between DJ-1 levels and clinical disease progression.

Methods

Materials

Reagents used were as follows: Tri-reagent (Sigma, St. Louis, MO, USA); rabbit anti-DJ-1 (Chemicon, Temecula, CA, USA); mouse anti-actin (Sigma, St. Louis, MO, USA); horseradish peroxidase conjugated goat anti-mouse and goat anti-rabbit (Sigma, St. Louis, MO, USA); Super Signal West Pico Chemiluminescent substrate (Pierce Biotechnology, Rockford, IL, USA); bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA); random primer (Invitrogen, Carlsbad, CA, USA); Sybr green polymerase chain reaction (PCR) master mix (Applied Biosystems, Warrington, UK); RNase inhibitor (RNAguard, Amersham Pharmacia biotech); Super Script II RNase H-reverse transcriptase (Invitrogen, Carlsbad, CA, USA); ready gel for polyacrylamide electrophoresis isoelectric focusing (IEF) pH 5–8 (Bio-Rad laboratories, Hercules, CA, USA); IEF 10× anode buffer (Bio-Rad laboratories, Hercules, CA, USA); IEF 10× cathode buffer (Bio-Rad Laboratories, Hercules, CA, USA); diethyl pyrocarbonate (DEPC) water (Biological Industries Israel Beit Haemek LTD, Kibbutz Beit Haemek, Israel); complete protease inhibitor cocktail tablets (Roche Diagnostics GmbH, Mannheim, Germany).

Other chemicals were purchased from Sigma Chemicals Co., St. Louis, MO, USA.

Animals

Mice overexpressing the human mutant superoxide dismutase ($SOD1^{G93A}$) were purchased from Jackson Laboratories. The $SOD1^{G93A}$ transgenic mouse model was developed and characterized as a model for amyotrophic lateral sclerosis by Gurney et al. (1994). The animals were housed in standard conditions: constant temperature (22±1°C), humidity (relative, 40%), and a 12-h light/dark cycle and were allowed free access to food and water. Male mice with hemizygous background were breeding with control females so that each litter would generate hemizygous $SOD1^{G93A}$ transgenic mice and littermate controls. At 1 month of age, offspring were genotyped by PCR analysis to confirm their transgenic status. The study was approved by the Tel-Aviv University ethical committee, and the mice were cared for and maintained in accordance with local and international regulations.

The 32 $SOD1^{G93A}$ transgenic mice and 20 wild-type age-matched littermates were killed at different ages and clinical disease severities. Specific stages of disease progression were evaluated in the $SOD1^{G93A}$ mice, specifically correlating with presymptomatic time point (age—35–45 days), onset of symptomatic muscle weakness (85–95 days), progressive disease with severe weakness but prior to paralysis (100–110 days), and end-stage disease (120–125 days). Brain and spinal cord tissues were frozen in liquid nitrogen and stored at −80°C until protein and RNA extractions.

Functional Motor Performance Test (Rotarod)

Mice were tested for their motor ability by rotarod examination using accelerating paradigm up to 16 rpm. Three trials were performed during the light phase of the 12-h light/12-h dark cycle for each mouse. The average duration was recorded. Time was stopped when the mouse fell from the rod or after an arbitrary limit of 120 s.

Protein Extraction and Western Blotting

Proteins were extracted from brain tissue by grinding in lysis buffer containing 250 mM sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 2 mM ethylene diamine tetraacetic acid, 20 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and protease inhibitors cocktail (Roche). Cell debris was removed by centrifugation at 20,000×*g* for 15 min at 4°C. Protein concentration was determined by the BCA method (Pierce). Twenty-five micrograms of total protein from brain samples lysate were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoreses gels and transferred to nitrocellulose membranes. The membranes were probed with rabbit anti-DJ-1 (1:5,000; Chemicon

Laboratories) and mouse anti beta-actin antibodies (1:10000; Sigma), followed by horseradish peroxidase conjugated secondary antibody (1:10000; Sigma) and developed with the Super Signal West Pico Chemiluminescent substrate (Pierce).

IEF

Proteins from SOD1^{G93A} transgenic and wild-type littermates brain extracts were separated in pH 5–8 ranges of isoelectric focusing phoresis gel (Bio-Rad) and transferred onto nitrocellulose membranes. Membranes were blotted with rabbit anti-DJ-1 antibodies (1:5000; Chemicon Laboratories), followed by horseradish peroxidase conjugated secondary antibody (1:10000; Sigma), and developed with the Super Signal West Pico Chemiluminescent substrate (Pierce).

RNA Isolation

Total RNA was isolated from mice brain tissues using a commercial reagent TriReagent™ (Sigma) and the manufacturer's recommended procedure. The amount of RNA was determined spectrophotometrically using the ND-1000 spectrophotometer (Nano-drop). RNA quality was verified by measuring OD260/OD280 ratio. RNA was stored at −80°C until used.

cDNA Synthesis

First-strand cDNA synthesis was carried out in a final reaction volume of 20 μl containing 1 μg of the total RNA, random primer (1.3 μM, Invitrogen UK) in DEPC-treated water at a total volume of 10 μl. After incubation in 70°C for 10 min and cooling to 4°C for 10 min, the following reagents were added to a final concentration: 1× buffer supplied by the manufacturer, 10 mM DTT, 20 μM dNTPs, 20 U of RNase inhibitor (RNAguard, Amersham Pharmacia biotech), and 10 U of the enzyme Super Script II RNase H-reverse transcriptase (Invitrogen). RT reaction was performed at 25°C for 10 min, 42°C for 2 h followed by 70°C for 15 min and 95°C for 15 min. Samples were stored at −20°C until used.

Real-time Quantitative Reverse Transcription PCR

Real-time quantitative PCR of the desired genes was performed in an ABI Prism 7700 sequence detection system (Applied biosystems) using Sybr green PCR master mix (Applied biosystems) and the following primers: DJ-1 sense TTA TCT GAG TCG CCT ATG GTG AAG, DJ-1 antisense GAG CCG TAG GAC CTG CAC AG, GAPDH sense CGA CAG TCA GCC GCA TCT T, GAPDH antisense CCA ATA CGA CCA AAT CCG TTG. *GAPDH* gene, which served as an internal control, is a valid reference "housekeeping" gene for transcription profiling.

For quantization of DJ-1 and GAPDH mRNA, real time quantitative PCR was performed in triplicates. The target gene, i.e. *DJ-1*, and the reference gene, *GAPDH*, PCR amplification was performed for each sample in separate wells of the same PCR reaction plate, which also contained standard curve for each gene-amplified and no template controls. Optimal experimental parameters (hybridization temperature, elongation time, and primers concentration) were determined for each primer pair. For each gene, verifying a single peak in melting curve analysis assessed the specificity of the PCR product.

The PCR was performed in a total volume of 20 μl containing 1 μl of the above-described cDNA, 1 μl each of the sense and antisense primers (final concentration of 500 nM each), 10 μl of Sybr Green Mix, and 7 μl of DEPC water.

The amplification protocol was 40 cycles of 95°C for 15 s followed by 60°C for 1 min each. Quantitative calculations of DJ-1 versus GAPDH was done using the $\Delta\Delta CT$ method, as instructed in the user bulletin #2 ABI prism 7700 sequence detection system (updated 10/2001).

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences software. We used student *t* test or one-way analysis of variance for comparisons between groups (as appropriate). Results are presented by mean ± standard error of the mean (SEM). Statistical significance is defined as $p \leq 0.05$.

Results

In order to evaluate possible involvement of DJ-1 in the motor neuron disease process, we evaluated DJ-1 mRNA and protein levels in the CNS of mutant SOD1^{G93A} transgenic mice as compared to wild-type littermates. Disease severity was evaluated using a functional motor test–rotarod examination. Mutant SOD1^{G93A} mice were killed at different disease stages (from asymptomatic to end stage disease) and wild-type littermates were killed at the same ages as controls, as described in "Methods."

Apparent clinical motor weakness was detected in SOD1^{G93A} mice from the age of 12 weeks (Fig. 1) with progressive weakness up to end stage at the age of 120–130 days. Surveillance of SOD1^{G93A} mice from our colony revealed mean survival of 139 days (129–153 days), which is in accordance with the known literature (Hensley et al. 2006).

We found that there was a significant elevation of DJ-1 mRNA levels in the brains of diseased SOD1^{G93A} transgenic

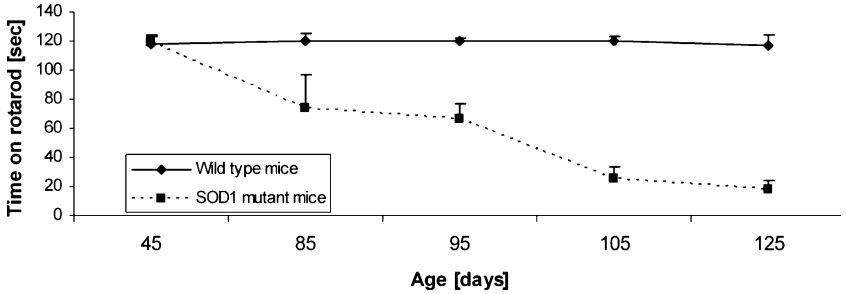

Figure 1 Progressive weakness of mutant SOD1^{G93A} transgenic mice as evident by accelerating rotarod test. Accelerating rotarod test was used as a quantitative tool in order to measure motor function of mutant SOD1^{G93A} mice. Time (measured in seconds, up to 120 s) until fall from an accelerating rotarod was measured for mutant SOD1^{G93A} mice and age-matched wild-type littermates. Each mouse was measured three times at each time point. Data is presented as mean ± SEM

mice, as compared to wild-type littermates ($p<0.05$; Fig. 2A). DJ-1 mRNA levels were significantly increased also in the spinal cords of SOD1^{G93A} transgenic mice as compared to wild-type littermates ($p<0.05$; Fig. 2B). For further evaluation, DJ-1 protein levels, extracted from diseased SOD1^{G93A} mice and control littermates, were assessed using Western blot. Upregulation of DJ-1 protein was noted in diseased SOD1^{G93A} transgenic mice, as compared to wild-type littermates ($p<0.05$; Fig. 3A,B).

It has been postulated that the pathogenesis of motor neuron disease in the mutant SOD1 fALS as well as in these transgenic mice involves an oxidative injury mechanism. Therefore, we sought to examine the changes in DJ-1 levels over their life span as compared to wild-type littermates. In order to investigate the timing of DJ-1 upregulation, we evaluated specific stages of disease progression in the SOD1^{G93A} mice, specifically correlating with a presymptomatic time point (age—35–45 days), the onset of symptomatic muscle weakness (85–95 days), progressive disease with severe weakness but prior to paralysis (100–110 days), and an end stage disease (120–125 days), as described in "Methods." Elevation of DJ-1 mRNA levels was noted throughout symptomatic disease stages. Moreover, elevation of DJ-1 mRNA was evident at an early disease stage in mildly symptomatic SOD1^{G93A} transgenic mice aging 12 weeks ($p<0.05$; Fig. 4). Similar to the elevation of DJ-1 mRNA, we found that upregulation of DJ-1 protein levels appeared at this early disease stage of mildly symptomatic SOD1^{G93A} transgenic mice aging 12 weeks, as shown by western blotting ($p<0.05$; Fig. 5A,B).

In order to detect oxidative modification in DJ-1 leading to accumulation of DJ-1 isoforms with more acidic pI, we used isoelectric focusing. Changes in the isoelectric point of DJ-1 isoforms were studied by isoelectric focusing phoresis gel with a range of pH 5–8. We found that in control brains, alkaline DJ-1 isoforms were the dominant DJ-1 isoform, while in SOD1^{G93A} mice, we demonstrate a shift towards the acidic isoforms (Fig. 6A,B). The shift toward acidic isoforms was more pronounced as disease progressed. Mild disease caused a slight change in the partition of DJ-1 isoforms. However, as the disease severity increased, in aged mutant SOD1^{G93A} mice exhibiting a more severe motor disability, a higher fraction of DJ-1 protein was composed from acidic DJ-1 isoforms. The partition of DJ-1

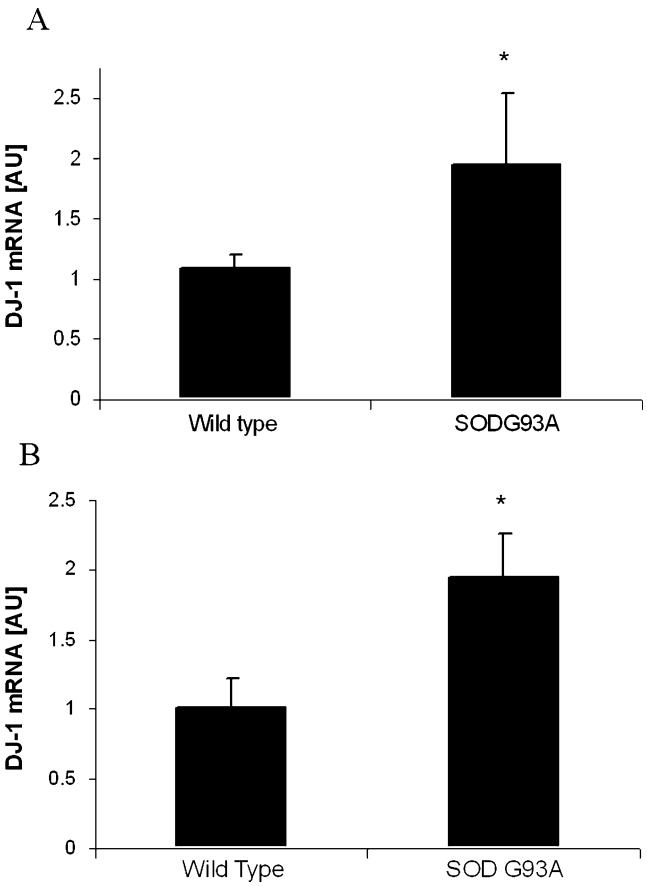

Figure 2 Elevated DJ-1 mRNA levels in brains and spinal cords of mutant SOD1^{G93A} transgenic mice. Diseased mutant SOD1^{G93A} transgenic mice were killed, and mRNA was extracted from the brains (**A**) and spinal cords (**B**). Real-time quantitative PCR for DJ-1 and *GAPDH*, which was used as a housekeeping gene, was performed as described in materials and methods. Quantitative calculations of DJ-1 versus GAPDH were done using the ddCT method. Upregulation of DJ-1 mRNA levels were detected in the brains and spinal cords of diseased mutant SOD1^{G93A} mice as compared to wild-type littermate controls. n=8–16/group. *$p<0.05$. Data is presented as mean ± SEM

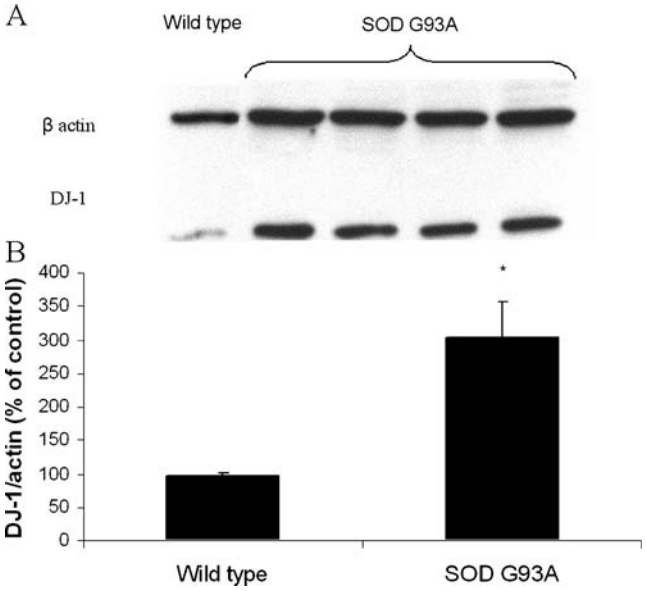

Figure 3 Elevated DJ-1 protein expression levels in brains of mutant SOD1^{G93A} transgenic mice. Diseased mutant SOD1^{G93A} transgenic mice were killed and DJ-1 protein levels were compared to age-matched wild-type littermates. Proteins were extracted from brain tissues, and DJ-1 expression was determined by Western blot analysis as described in materials and methods. **A** The membranes of Western blot were probed with rabbit anti-DJ-1 antibody (1:5000) and mouse anti beta-actin (1:10000). Beta-actin was used as an internal protein control. The *blot* presented here is from a representative experiment repeated three times with similar results. **B** Quantification of DJ-1 levels per beta-actin levels. n=8–16/ group. *p<0.05. Data is presented as mean ± SEM

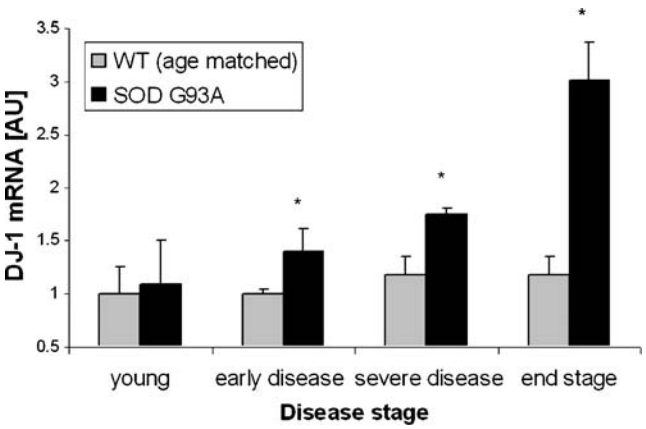

Figure 4 Elevated levels of DJ-1 mRNA in the central nervous system are evident in early symptomatic mutant SOD1^{G93A} transgenic mice. Symptomatic mutant SOD1^{G93A} transgenic mice were killed at different disease stages and compared to wild-type age-matched littermates. Real-time quantitative PCR for DJ-1 and GAPDH, used as a housekeeping gene, was performed as described. Significantly elevated levels of DJ-1 mRNA were detected in symptomatic SOD1^{G93A} transgenic mice, evident from early stage of the disease. n=4/group. *p<0.05. Data is presented as mean ± SEM

isoforms at end stage diseased transgenic mice, as compared to wild-type age-matched littermates, is presented at Fig. 6.

Discussion

This study demonstrated changes in DJ-1 expression levels and oxidized isoforms in brains and spinal cords of SOD1^{G93A} transgenic mice. We found that there is upregulation of DJ-1 mRNA and protein in SOD1^{G93A} transgenic mice as compared to wild-type littermates. Moreover, increased DJ-1 mRNA and protein levels appear at an early disease stage. We also detected an increase in DJ-1 acidic isoforms in diseased mice, implying that there are more oxidized forms of DJ-1 in the CNS of SOD1^{G93A} mice. The percentage of acidic DJ-1 isoforms of total DJ-1 protein increased with disease progression, implying on going oxidative damage. Collectively, the present findings strongly support the mechanistic concept that oxidative

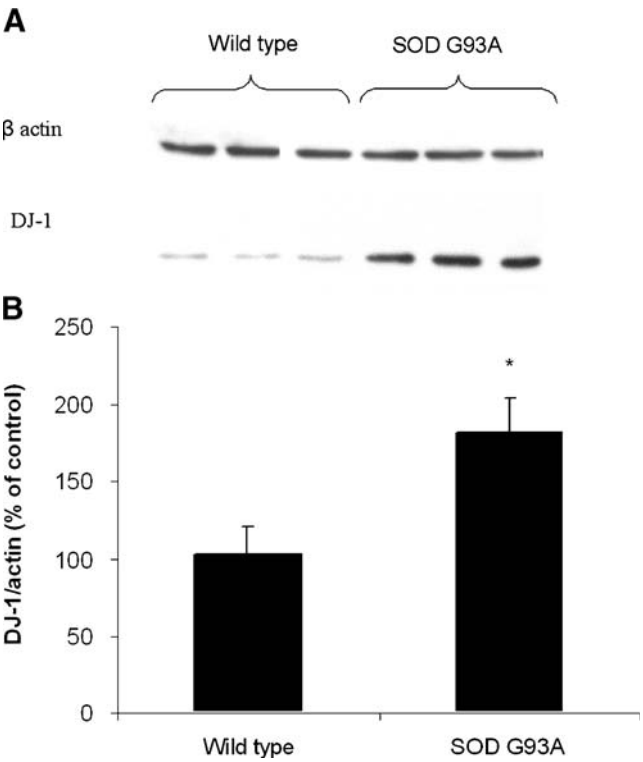

Figure 5 Upregulation of DJ-1 protein in the central nervous system is evident in early symptomatic mutant SOD1^{G93A} transgenic mice. Mildly symptomatic mutant SOD1^{G93A} transgenic mice, aged 12 weeks, were killed and the levels of DJ-1 protein were compared to wild-type littermates. **A** The membranes of Western blot were probed with rabbit anti-DJ-1 antibody (1:5000) and mouse anti beta-actin (1:10000), used as an internal protein control. The *blot* presented here is from a representative experiment repeated three times with similar results. **B** Quantification of DJ-1 protein levels per beta-actin levels is presented in the graph. *p<0.05. Data is presented as mean ± SEM

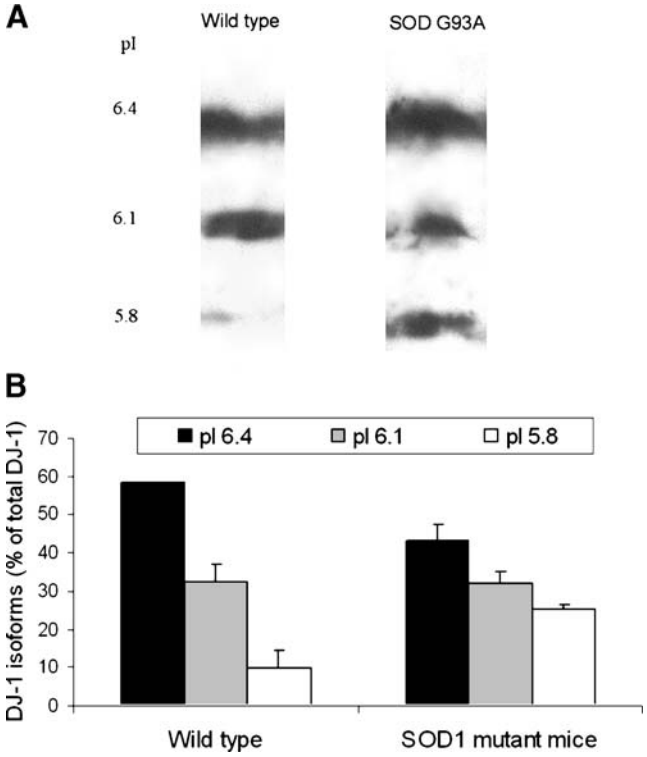

Figure 6 Shift toward acidic DJ-1 isoforms in diseased mutant $SOD1^{G93A}$ transgenic mice. Proteins were extracted from brain tissues of mutant $SOD1^{G93A}$ transgenic mice and wild-type littermates and DJ-1 pI was determined by IEF as described in materials and methods. pI shift of DJ-1 from basic isoforms to acidic forms was detected in mutant $SOD1^{G93A}$ transgenic mice as compared to wild type littermates. **A** Representative IEF probed with rabbit anti-DJ-1 antibody (1:5000) of end stage $SOD1^{G93A}$ transgenic mouse and a wild type littermate; **B** quantification of DJ-1 isoforms in end stage $SOD1^{G93A}$ transgenic mice and wild-type littermates (at the age of 4 months). Data is presented as mean ± SEM

injury to CNS participates in both the onset and progression of disease in $SOD1^{G93A}$ transgenic mice.

There is a large body of literature suggesting that an abnormal production of reactive oxygen species, leading to oxidative damage, contribute to the disease process in human ALS and in its transgenic mice models. Multiple studies demonstrated increased oxidative damage to proteins, lipids, and DNA in ALS post mortem tissues (Barber et al. 2006; Ilieva et al. 2007). Evidence for oxidative damage has been found in the spinal cord and in the motor cortex from sporadic ALS cases (Shaw et al. 1995; Fitzmaurice et al. 1996; Ferrante et al. 1997a; Shibata et al. 2007) and SOD1 familial ALS patients (Abe et al. 1995, 1997; Beal et al. 1997). Markers for protein and lipid oxidation were detected in motor neurons, reactive astrocytes, and microglia or macrophages (Shibata et al. 2001). Cerebrospinal fluid samples from ALS patients demonstrate increased levels of 8-OHdG (indicative of DNA oxidation), 4-hydroxynonenal (indicative of lipid peroxidation), ascorbate free radical, 3-nitrotyrosine levels and nitrated manganese superoxide dismutase (Smith et al. 1998; Tohgi et al. 1999; Aoyama et al. 2000; Bogdanov et al. 2000; Simpson et al. 2004; Ihara et al. 2005).

Transgenic mice models of ALS expressing mutant human SOD1 support the human studies showing increased oxidative damage to proteins, lipids, and DNA (Ferrante et al. 1997b; Andrus et al. 1998; Liu et al. 1998, 1999; Casoni et al. 2005; Poon et al. 2005). Protein oxidation and nitration have been reported in murine ALS (Andrus et al. 1998; Cha et al. 2000; Hensley et al. 2003, 2006). Protein carbonylation measured immunologically revealed an almost exponential increase in these posttranslational modifications between 90 and 120 days in the $SOD1^{G93A}$ mice (Andrus et al. 1998). Lipid oxidation products are likewise increased in ALS mice spinal cords (Hall et al. 1998; Perluigi et al. 2005; Shibata et al. 2007). Increased oxidative damage to DNA in spinal cord, cortex, and striatum of $SOD1^{G93A}$ mice was reported as well (Aguirre et al. 2005).

Accumulating evidence implies that DJ-1 plays an important role in the oxidative stress response. In vitro and in vivo studies suggest that vulnerability to oxidative insults is related to DJ-1 expression levels. DJ-1 knock-down by short-interfering RNA rendered neuroblastoma cells susceptible to hydrogen peroxide-, 1-methyl-4-phenyl-pyridinium-, rotenone, or 6-hydroxydopamine-induced cell death (Yokota et al. 2003; Taira et al. 2004; Sekito et al. 2006; Lev et al. 2008). Cell death induced by oxidative insults was dramatically reduced by overexpression of wild-type DJ-1 (Yokota et al. 2003; Kim et al. 2005; Lev et al. 2008). When primary cortical neurons, derived from brains of DJ-1+/+, DJ-1+/−, and DJ-1−/− mice embryos, were exposed to H_2O_2, DJ-1-deficient neurons showed a 20% increase in cell death compared to DJ-1+/+ neurons, while an intermediate amount of cell death was observed in DJ-1+/− neurons, suggesting a gene-dosage effect (Kim et al. 2005). In vivo studies confirmed that knock down of DJ-1 increased vulnerability to oxidative insults. DJ-1 knockout mice were more vulnerable to MPTP treatment, and restoration of DJ-1 expression by adenoviral vector delivery mitigated cell death (Kim et al. 2005). Drosophila possesses two homologous proteins of human DJ-1: DJ-1alpha and DJ-1beta. DJ-1alpha is expressed predominantly in the testis while DJ-1β is ubiquitously present in most tissues, resembling the expression pattern of human DJ-1 (Menzies et al. 2005). Loss-of-function DJ-1β mutants showed acute sensitivity to hydrogen peroxide treatment. However, a compensatory upregulation of DJ-1α expression in the brain that was sufficient to confer protection against paraquat insult was evident (Menzies et al. 2005). DJ-1α and DJ-1β double knockout flies displayed a striking sensitivity to paraquat and rotenone (Meulener et al. 2005). Moreover, we have recently shown that exposure of neuroblastoma cells to oxidative insults induced by the

neurotoxins rotenone and 6-hydroxydopamine led to rapid upregulation of DJ-1. Pretreatment with the antioxidant *N*-acetyl-cysteine abolished this upregulation, indicating that the upregulation of DJ-1 resulted from the oxidative stress (Lev et al. 2008). These results imply that DJ-1 has a role in the cellular defense mechanism against oxidative stress.

The observed upregulation of DJ-1 in diseased $SOD1^{G93A}$ transgenic mice, a well-established mice model of ALS, indicates that DJ-1 may be involved in the disease process in ALS. Since this is one of the protective mechanisms acting to overcome oxidative insults, upregulation of DJ-1 may serve as a protective mechanism in this disease. Similarly, antioxidant enzymes and redox-sensitive transcription factors, such as nuclear respiratory factor 1 and c-jun, were shown to elevate at early disease stages in SOD1 transgenic mice (Jokic et al. 2003; Mahoney et al. 2006)

Furthermore, upregulation of DJ-1 was noted at an early stage of the disease indicating that oxidative damage is early event acting in ALS. This is in accordance with previous studies that detected oxidative damage at early disease stages (Andrus et al. 1998; Mahoney et al. 2006; Hensley et al. 2006). The accumulation of oxidized isoforms of DJ-1 along disease progression proves that oxidative stress is an ongoing process throughout the progressive neurodegenerative process. This observation is also supported by Andrus et al. (1998) who detected an increase in protein carbonylation between 90 and 120 days in the $SOD1^{G93A}$ mice.

The mechanism of neuroprotection conferred by DJ-1 is still elusive. However, Martinat et al (2004) found that the initial accumulation of ROS is normal in DJ-1-deficient cells, but subsequent cellular defenses to ROS are impaired in DJ-1 deficient cells, leading to increased apoptosis and robustly increased protein carbonyl accumulation. One possible explanation is protection from mitochondrial oxidative damage. Oxidative insults result in translocation of DJ-1 into the mitochondria (Canet-Aviles et al. 2004; Lev et al. 2008). There is a substantial body of data that indicates that mitochondrial dysfunction is a feature of motor neuron degeneration in ALS (Barber et al. 2006). Mutant SOD1 has also been found within mitochondria, and such accumulation of mutant SOD1 preceded the onset of symptoms in SOD1 transgenic mice and coincided with increased oxidative damage (Liu et al. 2004). As the major source of reactive oxygen species, mitochondria are implicated in production of oxidative stress and are also targets of ROS. One possible explanation is that translocation of DJ-1 into the mitochondria might serve to protect them against oxidative damage.

Another possible explanation for neuroprotection conferred by DJ-1 in ALS is DJ-1's effect on transcription of neuroprotective and detoxifying genes. The antioxidant response element (ARE) is an enhancer element that initiates the transcription of a battery of genes encoding phase II detoxification enzymes and factors essential for neuronal survival (Kraft et al. 2007). The ARE is activated through heterodimeric DNA binding of its transcription factor, NFE2-related factor 2 (Nrf2; Kraft et al. 2007). Kraft et al. (2007) found activation of the Nrf2–ARE pathway throughout the time course of motor neuron disease in $SOD1^{G93A}$ mice. Clements et al (2006) found that DJ-1 stabilizes Nrf2 by preventing its association with its inhibitor protein, Keap1, and Nrf2's subsequent ubiquitination. Without intact DJ-1, Nrf2 protein is unstable, and transcriptional responses are, thereby, decreased both basally and after oxidative induction.

Further research into the functional mechanisms activated by DJ-1 is needed in order to elucidate its exact role in ALS. However, upregulation of DJ-1 in a model of ALS, which was described here for the first time, can serve as a novel target for therapeutic intervention as well as a mean to follow the disease activity in ALS patients.

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