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Review article

Cytoprotective mechanisms of DJ-1 against oxidative stress through modulating ERK1/2 and ASK1 signal transduction

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ABSTRACT

DJ-1 is a highly conserved multifunctional protein linked to both neurodegeneration and neoplasia. Among its various activities is an antioxidant property leading to cytoprotection under oxidative stress conditions. This is associated with the ability to modulate signal transduction events that determine how the cell regulates normal processes such as growth, senescence, apoptosis, and autophagy in order to adapt to environmental stimuli and stresses. Alterations in DJ-1 expression or function can disrupt homeostatic signaling networks and initiate cascades that play a role in the pathogenesis of conditions such as Parkinson's disease and cancer.

DJ-1 plays a major role in various signaling pathways. Related to its anti-oxidant properties, it mediates cell survival and proliferation by activating the extracellular signal-regulated kinase (ERK1/2) pathway and attenuates cell death signaling by inhibiting apoptosis signal-regulating kinase 1 (ASK1) activation. Here, we review the ways through which DJ-1 regulates these pathways, focusing on how its regulation of signal transduction contributes to cellular homeostasis and the pathologic states that result from their dysregulation.

1. Introduction

DJ-1 is a highly conserved, homodimeric protein that was originally cloned as an oncogene capable of transforming cells in cooperation with activated *ras* [1]. DJ-1 is over-expressed in multiple tumor types and is positively correlated with tumor metastasis and negatively correlated with patient survival [2–8]. Knockdown of DJ-1 sensitizes various tumor cell types to chemotherapeutic drugs [7], demonstrating its crucial role in tumor maintenance. Increased levels of DJ-1 in serum and extracellular fluids have also been proposed as a predictive biomarker in some cancers [9,10] highlighting its potential for cancer diagnosis and prognosis.

While over-expression of DJ-1 in somatic cell lines appears to mediate cancer development, loss of function mutations of DJ-1 in post-mitotic neurons are linked to recessively inherited Parkinson's disease characterized by neuodegeneration of substantia nigra dopaminergic neurons [11]. This places DJ-1 at the center of a nuanced balance where it can regulate cellular processes depending on cell type and serves as a determinant of cell survival or cell death in response to extracellular stimuli.

One way that DJ-1 appears to control cellular homeostasis is through its ability to modulate signal transduction - cell signaling pathways which are able to convey, amplify, and translate the information transmitted from the plasma membrane to the nucleus. For example, DJ-1 can activate the extracellular signal-regulated kinase (ERK1/2) pathway [12,13] and the phosphatidylinositol-3-kinase (PI3K)/Akt pathway [8] to mediate cell survival and proliferation. It can attenuate cell death signaling by inhibiting apoptosis signal-regulating kinase 1 (ASK1) activation [14,15] as well as the mitogen-activated protein kinase kinase kinase 1 (MEKK1/MAP3K1) activation [16] of downstream apoptotic cascades. It also modulates autophagy through many signaling pathways [17–19], a process that can mediate either cell survival or cell death depending on the circumstances [20].

The direct neuroprotective effects of DJ-1 have long been attributed to cysteine residues that sense and attenuate oxidative stress. Its three cysteine residues at Cys46, Cys56, and Cys106 are thought to scavenge reactive oxygen species (ROS) with the quenching activity of their sulfhydryl groups, thereby reducing cellular ROS burden [21]. Cys106, the critical residue considered most susceptible to oxidation, is oxidized to cysteine sulfenic acid (Cys-SOH), cysteine sulfinic acid (Cys-SO_2H), and then cysteine sulfonic acid (Cys-SO_3H) forms, causing the isoelectric point (pI) to shift towards more acidic values. Excessively oxidized Cys106-SO_3H form of DJ-1 is considered inactive, and mutation of Cys106 results in loss of neuroprotective function [22]. The antioxidant activity of DJ-1 is demonstrated by its ability to protect neurons against toxins that increase cellular ROS levels, including H_2O_2, 6-

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OHDA, rotenone and MPTP, in various *in vitro* and *in vivo* studies [18,23–25]. Conversely, knocking down DJ-1 exacerbates the cell death induced by oxidative stress [25–29].

Investigating the function of DJ-1 in cell signaling has been helpful in understanding its function in maintaining cellular homeostasis, and its contrasting roles in neurodegeneration and cancer [30,31]. In this review, we focus on the extracellular signal-regulated kinase (ERK1/2) pathway and the Daxx-apoptosis signal-regulating kinase 1 (ASK1) death signaling pathway as they relate to the anti-oxidant activity of DJ-1.

1.1. Activation of the ERK1/2 pathway by DJ-1

The extracellular signal-regulated kinase (ERK1/2) pathway is a classic mitogen-activated protein kinase (MAPK) signaling cascade that regulates cell proliferation, growth, autophagy, and differentiation. Core pathway members include Ras, Raf, MEK1/2, and ERK1/2 (MAPK) [32,33]. Various stimuli activate this pathway, including growth factors, polypeptide hormones, neurotransmitters, chemokines, and phorbol esters, which bind or activate a variety of receptors and proteins such as receptor tyrosine kinases (RTKs), G-protein coupled receptors (GPCRs), and protein kinase C (PKC) [34].

ERK1/2 are serine-threonine kinases that are positively regulated by MEK1/2-mediated phosphorylation. MEK1/2 are MAPKK proteins with ERK1/2 as their only known physiological substrates [35]. On the other hand, ERK is negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases (DUSPs/MKPs) [36]. Activated ERK phosphorylates several downstream transcription factors such as AP-1, c-Jun and c-Myc [37]. ERK can also activate Ribosome S6 Kinase (RSK) and inhibitor kappa-β Kinase (IKK), which can lead to the respective activation of transcription factors cAMP Response Element-Binding (CREB) and Nuclear Factor immunoglobulin Kappa-chain enhancer-β-cell (NF-kappa-β) [35,38].

Several studies have shown that DJ-1 activates ERK1/2, a phenomenon that can contribute to many of the roles that DJ-1 plays in protecting cells from oxidative injury and in regulating gene transcription (Fig. 1).

1.1.1. DJ-1 protects against oxidative injury by activating ERK1/2 and MEK1/2

Several studies have demonstrated that DJ-1 functions upstream of ERK1/2 phosphorylation [12,39–41]. For example, over-expression of wild-type (WT) DJ-1 in COS-7 or MN9D cells up-regulates ERK1/2 and MEK1/2 phosphorylation, while L166P mutant DJ-1, which is linked to Parkinson's disease, cannot enhance ERK1/2 or MEK1/2 phosphorylation [12]. In cancer cell models (T47-D and MCF-7), knock-down of DJ-1 using shRNA leads to down-regulation of phosphorylated ERK1/2 and decreased cell proliferation [42].

In a variety of models, it is well-established that DJ-1 over-expression improves the viability of cells challenged with hydrogen peroxide (H_2O_2) [43,44]. Under these conditions, inhibiting ERK1/2 activation by pretreating cells with the MEK1/2 inhibitor U0126 abolishes the protective effect of WT DJ-1 over-expression. This observation suggests that DJ-1 can protect cells from oxidative injury through activation of the ERK pathway [12].

1.1.2. DJ-1 regulates dopamine (DA) homeostasis through activation of ERK1/2 and the nuclear translocation of Nurr1

One way that DJ-1 may mediate this protective effect is through modulation of Nurr1 levels. The transcription factor Nurr1 plays a major role in dopamine homeostasis. It regulates the expression of dopamine synthetic enzymes tyrosine hydroxylase (TH) and L-dopa decarboxylase (DDC), as well as the expression of vesicular monoamine transporter 2 (VMAT-2), which is necessary for the transport of DA from the cytosol into synaptic vesicles [45–47]. In both *in vivo* and *in vitro* models, DJ-1 has been shown to modulate Nurr1. Over-expression of WT DJ-1 in MN9D cells leads to an increase in the nuclear translocation of Nurr1 as well as an increase in the mRNA levels of Nurr1 target genes. The Parkinson associated pathogenic L166P mutant form of DJ-1, on the other hand, cannot impact Nurr1. As expected, knocking-down DJ-1 expression attenuates the activity of Nurr1 and down-regulates the expression of its target genes [48].

Considering that ERK1/2 increases Nurr1 transcriptional activity [49], it was hypothesized that DJ-1 may regulate Nurr1 activation through the ERK1/2 pathway. Indeed, over-expression of WT DJ-1, but not its L166P mutant, leads to phosphorylation of ERK1/2, and blocking ERK1/2 activation using U0126 prevents DJ-1-mediated nuclear translocation of Nurr1 and the induction of Nurr1 target genes [50]. A similar phenomenon has been shown *in vivo*. Over-expression of WT DJ-1 but not its L166P mutant in the substantia nigra of rats using a lentiviral vector increases ERK activation, Nurr1 nuclear translocation, as well as Nurr1 target protein levels [50].

1.1.3. DJ-1 interacts genetically with ERK1/2

DJ-1 has been shown to interact directly with ERK1/2 [41], and to modulate upstream factors in the MAPK cascade, either through direct interaction or by affecting protein expression [12,13,40].

Mice lacking both DJ-1 and the Glial Neurotrophic Factor (GDNF) tyrosine kinase receptor Ret lose more dopaminergic neurons in the substantia nigra compared to mice lacking only Ret, suggesting a possible cooperation between DJ-1 and Ret [51]. As Ret is upstream of the ERK pathway and necessary for the neuronal survival activity of GDNF [52], a developing *Drosophila* system has been employed to study the effect of DJ-1 on Ret signaling and downstream MAPK pathways. Flies that were made to over-express constitutively active Ret exhibited developmental abnormalities but had unaltered endogenous DJ-1 levels. When these flies were crossed with *Drosophila* expressing reduced DJ-1 levels, the offspring showed complete rescue of the abnormal phenotype. Conversely, when flies expressing constitutively active Ret were crossed with *Drosophila* over-expressing DJ-1, the offspring exhibited more severe developmental defects. These findings indicate a genetic interaction between Ret and DJ-1 in controlling cell size and differentiation [51]. Similarly, DJ-1 interacts genetically with Ras and with ERK/*rolled* (rl), suggesting that DJ-1 may cooperate with Ret, Ras, and ERK during development to control cell differentiation and proliferation [51].

1.1.4. DJ-1 interacts directly with and affects the nuclear translocation of ERK1/2

The transcription factor Elk1 binds to the promoter of the superoxide dismutase (SOD) gene and enhances its expression leading to reduced ROS generation [53]. Elk1 is phosphorylated and activated by MAPK kinases such as ERK1/2 [54], suggesting that one of the mechanisms through which DJ-1 protects cells against ROS is *via* ERK1/2-Elk1 activation leading to SOD induction. Notably, Elk1 activation in the substantia nigra of mice challenged with the mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is blunted in DJ-1 KO mice compared to WT mice [41]. However, in contrast to *in vitro* data showing that knocking down DJ-1 reduces ERK1/2 phosphorylation [48], ERK1/2 phosphorylation is not affected in DJ-1 KO mouse brains [41]. This has led to the hypothesis that under oxidative insult, DJ-1 may act as a molecular chaperone [55], to affect the nuclear translocation of ERK1/2 rather than its phosphorylation.

Evidence for direct interaction between DJ-1 and ERK2 has been presented in HEK293T cells and in mouse brain lysates using co-immunoprecipitation [41]. This interaction involves residues 1–100 of DJ-1, but is not impacted by mutation of its cysteine 106, which is necessary for its role as a redox sensor and peroxide scavenger [21,56,57]. Additionally, the nuclear translocation of ERK1/2 is reduced in DJ-1 knock down SH-SY5Y cells and in DJ-1 KO primary mouse neurons [41]. This suggests that DJ-1 promotes the translocation of ERK1/2 to the nucleus upon oxidative stress, allowing ERK1/2 to phosphorylate/