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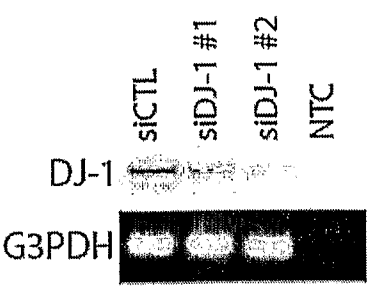
(54) Title: METHODS AND COMPOSITIONS DIRECTED TO DJ-1 AS REGULATOR OF THE ANTI-OXIDANT TRANSCRIPTION FACTOR NRF2



(57) Abstract: The present invention provides compositions and methods related to the field of oxidative stress in cells. The present invention provides compositions and methods for manipulating and assessing DJ-1 and Nrf2 activity, thereby affecting an oxidative stress response. The present invention also provides methods for treating diseases and disorders associated with an altered oxidative stress response.

METHODS AND COMPOSITIONS DIRECTED TO DJ-1 AS REGULATOR OF THE ANTI-OXIDANT TRANSCRIPTION FACTOR NRF2

STATEMENT OF FEDERALLY FUNDED RESEARCH SUPPORT

Certain aspects of this invention were supported by Grant No. CA58223 awarded by the National Cancer Institute. The U.S. Government has certain rights in this invention.

RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 60/697,606, filed July 8, 2005, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention provides compositions and methods related to the field of oxidative stress in cells. More specifically, the present invention provides compositions and methods for manipulating and assessing DJ-1 and Nrf2 activity in a cell and thus modulating a cell's oxidative stress response. The present invention also provides methods for treating a disease or disorder associated with DJ-1 and/or Nrf2 activity in a cell.

BACKGROUND OF THE INVENTION

Oxidative stress has been implicated as a major contributing factor to a wide variety of ailments. Cancer, cardiovascular disease, neurodegenerative disorders, and aging are all associated with increased oxidative stress in tissues. Such stress results from the accumulation of oxidative species due to their metabolic generation and environmental exposures. These oxidative species are detoxified by anti-oxidant enzymes and molecules. The balance between oxidative species generation and removal determines the oxidative stress on a given tissue. Not surprisingly therefore, cellular responses to oxidative stress are major determinants of disease susceptibility. This is particularly true in tissues that are sensitive to oxidative stress such as the central nervous system. Genetic defects in oxidative responses lead to neurodegenerative diseases.

The transcription factor Nuclear Factor Erythroid 2-like 2 (Nrf2) is a master regulator of response to oxidative stress. Nrf2 is a member of the Cap 'n' Collar (CNC) family of b-Zip transcription factors that regulate the expression of many anti-oxidant pathway genes (HY Cho, et al. *Antioxid Redox Signal* (2006) 8:76-87). In the absence of toxic insult, Nrf2 is

maintained at basal levels in cells bound to its inhibitor protein, Kelch-like Ech-Associated Protein 1 (KEAP1) (K Itoh, et al. *Genes Dev* (1999) 13:76-86; S Dhakshinamoorthy, et al. *Oncogene* (2001) 20:3906-17). KEAP1 targets Nrf2 for ubiquitination leading to its constitutive degradation (SB Cullinan, et al. *Mol Cell Biol* (2004) 24:8477-86; A Kobayashi, et al. *Mol Cell Biol* (2004) 24:7130-9). Upon exposure to oxidative stress, xenobiotics, or electrophilic compounds, the Nrf2 protein is stabilized and translocates to the nucleus (C Chen, et al. *Free Radic Biol Med* (2004) 36:1505-16). There it forms heterodimers with other transcription regulators, such as small Maf proteins, and induces the expression of anti-oxidant genes (K Itoh, et al. *Biochem Biophys Res Commun* (1997) 236:313-22; AC Wild, et al. *J Biol Chem* (1999) 274:33627-36). Nrf2 drives the expression of detoxification enzymes such as NQO1, Hmox-1, and enzymes that generate anti-oxidant molecules such as glutathione (R Venugopal, et al. *Proc Natl Acad Sci U S A* (1996) 93:14960-5; J Alam, et al. (1999) *J Biol Chem* 274:26071-8). Nrf2 function and the expression of its regulated genes have been implicated in the risk and/or prevention of both cancer and Parkinson's disease (M Ramos-Gomez, et al. *Proc Natl Acad Sci U S A* (2001) 98:3410-5; MK Kwak, et al. *J Biol Chem* (2003) 278:8135-45; K Nakaso, et al. *Biochem Biophys Res Commun* (2006) 339:915-22).

DJ-1 is a gene that has been associated with Parkinson's disease. Loss of DJ-1 has been found to lead to early onset Parkinson's disease with high penetrance (V Bonifati, et al. *Science* (2003) 299:256-9). Furthermore, DJ-1 expression in cancer cell lines has been shown to convey protection against stresses including chemotherapy, oxidative stress, ER stress, and proteosome inhibition (JP MacKeigan, et al. *Cancer Res* (2003) 63:6928-34; T Taira, et al. *EMBO Rep* (2004) 5:213-8; T Yokota, et al. *Biochem Biophys Res Commun* (2003) 312:1342-8).

The present invention is based on the discovery that DJ-1 is required for the activity of Nrf2. DJ-1 is indispensable for Nrf2 protein stabilization in multiple cell types, and alters the ubiquitination of Nrf2. Over-expression of DJ-1 prevents the ubiquitination of Nrf2 and disrupts the Nrf2-KEAP1 interaction. DJ-1 is required for the expression of several genes including the prototypic Nrf2 regulated anti-oxidant enzyme NAD(P)H Quinone Oxidoreductase I (NQO1). Furthermore, reconstitution of DJ-1 deficient cells partially restores Nrf2 function. These findings support the importance of DJ-1's effects on Nrf2 in the development of Parkinson's disease and cancer. Thus, the present invention provides methods and compositions directed to the association between DJ-1 and Nrf2 and modulation

of activities that involve such an association, such as in the treatment of disorders associated with an altered oxidative stress response.

SUMMARY OF THE INVENTION

The present invention provides a method of identifying a compound having the ability to modulate an anti-oxidant response directed by DJ-1 or Nrf2, comprising: a) contacting the compound with DJ-1 and Nrf2 under conditions whereby an anti-oxidant response can occur; and b) determining the amount or effect of the anti-oxidant response, whereby a decrease or increase in the amount and/or effect of the anti-oxidant response in the presence of the compound as compared to the amount and/or effect of the anti-oxidant response in the absence of the compound identifies a compound having the ability to modulate an anti-oxidant response directed by DJ-1 or Nrf2.

In an additional embodiment, the present invention provides a method of identifying a compound having the ability to modulate the production of DJ-1, comprising: a) contacting the compound with a cell that produces DJ-1; and b) determining the amount of DJ-1 mRNA and/or the amount of DJ-1 protein produced in the cell, whereby an increase or decrease in the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the presence of the compound as compared to the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the absence of the compound identifies a compound having the ability to modulate production of DJ-1.

In a further embodiment, the present invention provides a method of identifying a compound having the ability to modulate the activity of DJ-1, comprising: a) contacting the compound with a cell in which DJ-1 has activity; and b) determining the amount of DJ-1 activity in the cell, whereby an increase or decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the activity of DJ-1.

In another embodiment, the present invention provides a method of identifying a compound having the ability to modulate the production of Nrf2 by modulating DJ-1 activity, comprising: a) contacting the compound with a cell in which DJ-1 has activity; and b) determining the amount of DJ-1 activity in the cell, whereby an increase of decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the production of Nrf2 by modulating DJ-1 activity.

In another embodiment, the present invention provides a method of identifying a compound having the ability to modulate the activity of Nrf2 by modulating DJ-1 activity, comprising: a) contacting the compound with a cell in which DJ-1 has activity; and b) determining the amount of DJ-1 activity in the cell, whereby an increase or decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the activity of Nrf2 by modulating DJ-1 activity.

In another embodiment, the present invention provides a method of identifying a gene involved in an oxidative stress response, wherein the transcription of said gene is regulated by Nrf2, in a cell, comprising: a) contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby reducing Nrf2 activity in the cell; and b) identifying a gene having altered transcription in the cell of step (a), thereby identifying a gene involved in the oxidative stress response of the cell.

In another embodiment, the present invention provides a method of modulating an anti-oxidant response in a cell, wherein the anti-oxidant response is directed by DJ-1 or Nrf2, comprising contacting the cell with a compound that alters DJ-1 activity in the cell, thereby altering Nrf2 activity in the cell and modulating the anti-oxidant response in the cell.

In another embodiment, the present invention provides a method of modulating DJ-1 activity in a cell, comprising contacting the cell with a compound that modulates DJ-1 activity in the cell.

In another embodiment, the present invention provides a method of modulating Nrf2 activity in a cell, comprising contacting the cell with a compound that modulates DJ-1 activity in the cell.

In another embodiment, the present invention provides a method of identifying a subject as having a disorder associated with an altered oxidative stress response, comprising: a) measuring an amount of DJ-1 activity in a cell of the subject; and b) comparing the amount of DJ-1 activity in the cell of (a) with the amount of DJ-1 activity in a reference cell, whereby an increased or decreased amount of DJ-1 activity in the cell of (a) as compared to the amount of DJ-1 activity in the reference cell identifies a subject as having a disorder associated with an altered oxidative stress response.

In another embodiment, the present invention provides a method of identifying a subject with a disorder associated with an altered cellular oxidative stress response as having a poor prognosis, comprising: a) measuring an amount of DJ-1 activity in a cell of the subject; and b) comparing the amount of DJ-1 activity in the cell of (a) with the amount of

DJ-1 activity in a reference cell, whereby an increased or decreased amount of DJ-1 activity in the cell of (a) as compared to the amount of DJ-1 activity in the reference cell identifies a subject with a disorder associated with an altered oxidative stress response as having a poor prognosis.

In another embodiment, the present invention provides a method of identifying a compound useful for treating a disorder associated with an altered oxidative stress response, comprising: a) contacting the compound with DJ-1 under conditions whereby DJ-1 activity can be measured; and b) measuring the amount of DJ-1 activity of (a); and c) comparing the amount of DJ-1 activity of (b) with the amount of DJ-1 activity in the absence of the compound, whereby a decrease or increase in the amount of DJ-1 activity in the presence of the compound as compared to the amount of DJ-1 activity in the absence of the compound identifies a compound useful for treating a disorder associated with an altered oxidative stress response.

In another embodiment, the present invention provides a method of treating a subject having a disorder associated with an altered oxidative stress response, comprising administering to the subject an effective amount of a compound that modulates DJ-1 activity, thereby modulating Nrf2 activity and treating the disorder associate with an altered oxidative stress response.

In another embodiment, the present invention provides a method of identifying a compound that decreases the amount of DJ-1 in a cancer cell in which DJ-1 is produced, comprising: a) contacting the compound with the cancer cell; and b) measuring the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell of (a), whereby a decrease in the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the presence of the compound as compared to the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the absence of the compound identifies a compound that decreases the amount of DJ-1 in the cancer cell in which DJ-1 is produced.

In another embodiment, the present invention provides a method of identifying a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced, comprising:

a) contacting the compound with the cancer cell; and

b) determining the amount of DJ-1 activity in the cancer cell of (a), whereby an increase or decrease in the amount of DJ-1 activity in the cancer cell in the presence of the compound as compared to the amount of DJ-1 activity in the cancer cell in the absence of the

compound identifies a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced.

In another embodiment, the present invention provides a method of identifying a cancer cell having increased resistance to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to the amount of DJ-1 and/or Nrf2 activity in a non-cancer cell identifies a cancer cell having an increased resistance to a chemotherapeutic agent.

In another embodiment, the present invention provides a method of identifying a cancer cell that is susceptible to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to a non-cancer cell identifies a cancer cell that is susceptible to a chemotherapeutic agent.

In another embodiment, the present invention provides a method of treating a cancer associated with an altered oxidative response in a subject, comprising administering to the subject an effective amount of a compound that modulates DJ-1 activity, thereby modulating Nrf2 activity and treating the cancer associated with an altered oxidative stress response.

In another embodiment, the present invention provides a method of identifying a chemotherapeutic agent having a therapeutic effect in a cancer cell, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent, whereby a decrease in the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent as compared to the amount of DJ-1 and/or Nrf2 activity in the absence of the chemotherapeutic agent identifies a chemotherapeutic agent having a therapeutic effect in the cancer cell.

In another embodiment, the present invention provides a method of identifying a chemotherapeutic agent that is resistant to Nrf2- mediated detoxification, comprising: a) contacting the chemotherapeutic agent with a cell under conditions whereby an Nrf2-mediated detoxification response can occur; and b) determining if the chemotherapeutic agent has a therapeutic effect on the cell, whereby a chemotherapeutic agent that has a therapeutic effect on the cell in the presence of the Nrf2-mediated detoxification response identifies a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification.

In another embodiment, the present invention provides a method of treating a cancer in a subject, wherein the cancer is associated with an altered oxidative stress response by inhibiting an anti-oxidant response of an Nrf2-regulated gene, comprising administering to

the subject an effective amount of a compound that reduces DJ-1 activity in the cell, thereby reducing Nrf2 activity in the cell, resulting in the downregulation of expression of the Nrf2-regulated gene and an inhibition of the anti-oxidant response of the gene.

In another embodiment, the present invention provides a method of treating Parkinson's disease in a subject whose cells are deficient in DJ-1 and/or Nrf2 function by inducing expression of Nrf2-regulated genes, comprising administering to the subject an effective amount of an exogenous nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence is expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

In another embodiment, the present invention provides a method of protecting neurons from destruction in a subject with Parkinson's disease, wherein the cells of the subject are deficient in DJ-1 and/or Nrf2 function, by inducing expression of Nrf2-regulated genes in the subject, comprising administering to the subject an effective amount of an exogenous nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence is expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

In another embodiment, the present invention provides a method of downregulating the expression of an Nrf2-regulated gene that is associated with an anti-oxidant response in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity, thereby reducing Nrf2 activity, resulting in the downregulation of expression of the Nrf2-regulated gene.

In another embodiment, the present invention provides a method of upregulating the expression of an Nrf2-regulated gene that is associated with an anti-oxidant response in a cell, comprising contacting the cell with a nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence can be expressed to produce DJ-1 protein, whereby the amount of DJ-1 in the cell is increased, resulting in an increase in the amount of Nrf2 in the cell and upregulation of expression of the Nrf2-regulated gene.

In another embodiment, the present invention provides a method of identifying a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2, comprising: a) contacting a fragment comprising at least ten contiguous amino acids of the amino acid sequence of the DJ-1 protein with Nrf2 and KEAP1 under conditions whereby binding between Nrf2 and KEAP1 can occur; and b) determining the amount of Nrf2 bound to

KEAP1 in the presence of the fragment as compared to the amount of Nrf2 and KEAP1 bound in the absence of the fragment, whereby a decrease in the amount of Nrf2 bound to KEAP1 in the presence of the fragment as compared to the amount of Nrf2 bound to KEAP1 in the absence of the fragment identifies a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2.

In another embodiment, the present invention provides a method of identifying a compound that modulates the binding of DJ-1 to a protein selected from the group consisting of amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4 (ALS2CR4), silent mating type information regulation 2 homolog 7 (sirtuin 7), WD repeat domain 5 (WDR5), plasminogen activator inhibitor 1 RNA binding protein (PAI1RBP; also known as PAI-1 mRNA binding protein and chromodomain helicase DNA binding protein 3 interacting protein), eukaryotic translation initiation factor 2, subunit 2 beta, 38 kDa (EIF2S2) and any combination thereof.

In another embodiment, the present invention provides a method of identifying a compound that modulates the binding of DJ-1 to a protein in the presence of an oxidative stress (e.g., oxidizing H_2O_2), wherein the protein is selected from the group consisting of proapolipoprotein A1, haptoglobin Hp2, lipoprotein CIII, alpha-1-antitrypsin (aa 268-394), amyloid fibril protein-transthyretin-related, glyceraldehydes-3-phosphate dehydrogenase, ADP/ADT translocator protein, human serum albumin in a complex with myristic acid and tri-iodobenzoic acid, ATP-binding cassette, sub family A, member 3, complement component 3 precursor, hypothetical protein MGC20781, transferrin, heat shock 70 kDa protein 8 isoform 1, hypothetical protein LOC345651, ADP/ATP carrier protein, carbamyl phosphate synthetase I and any combination thereof.

In another embodiment, the present invention provides a method of measuring the efficacy of a chemotherapeutic agent for treating a cancer in a subject, comprising: a) measuring the amount of DJ-1 activity in the subject before administering the chemotherapeutic agent to the subject; b) administering the chemotherapeutic agent to the subject; c) measuring the amount of DJ-1 activity in the subject during and/or after administering the chemotherapeutic agent to the subject; and d) comparing the amount of DJ-1 activity of (a) with the amount of DJ-1 activity of (c), whereby a decrease in the amount of DJ-1 activity of (c) identifies a chemotherapeutic agent having efficacy for treating the cancer in the subject.

In another embodiment, the present invention provides a method of downregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a

compound that reduces DJ-1 activity in the cell, thereby downregulating expression of the gene.

In another embodiment, the present invention provides a method of upregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby upregulating expression of the gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-C. siRNA mediated knockdown of DJ-1. A. End-point RT-PCR of siRNA transfected H157 cells. DJ-1 is presented in negative so bands can be more easily visualized. B. Western blot analysis of siRNA transfected H157 cells demonstrating DJ-1 knockdown at the protein level. C. Quantitative real-time Q-PCR of DJ-1 mRNA following siRNA transfection (relative mRNA quantitation normalized to 18S rRNA expression; siDJ-1 #2 knocks down DJ-1 expression to a greater degree than siDJ-1 #1, while transfection with either a scrambled non-specific oligomer siRNA or transfection reagent alone (siMock) does not affect DJ-1 expression).

Figures 2A-D. Measurements of the effect of DJ-1 on Nrf2 mRNA expression and Nrf2 mediated transcription of other genes. A. Luciferase reporter gene activity in Huh7 cells following siRNA transfection. The firefly luciferase reporter construct is under the control of the NQO1 anti-oxidant response element (ARE), which is responsive to Nrf2. Cells were then treated with 50 μM tBHQ or a DMSO vehicle control. Lysates were assayed for luciferase activity and normalized to crude protein present in the extract. Flag-Nrf2 was transfected as a positive control. Samples with lowered DJ-1 expression contained lower levels of the reporter enzyme and failed to induce following treatment with tBHQ. B. Luciferase activity expressed from a construct under the control of the constitutively active viral SV40 promoter. C. Specific activation of luciferase reporter constructs. siRNA transfected Huh7 cells were further transfected with luciferase reporter constructs under control of either the NQO1 ARE [ARE], glucocorticoid response element [GRE], or c-AMP response element [CRE]. Cultures were treated with either the appropriate vehicle control or 50 μM tBHQ, 100 μM dexamethasone, or 10 μM forskalin respectively. Activation is presented as the percent induction of control oligomer (siCTL) transfected cells. D. Real-time Q-PCR analysis of mRNA expression following siRNA knockdown of DJ-1 verifies that NQO1 expression is decreased following DJ-1 knockdown. Nrf2 was unaffected by the loss of DJ-1 at the mRNA level.

Figures 3A-D. Protein analysis establishing that DJ-1 is required for Nrf2 protein stability. A. Western blot analysis of Huh7 cell lysates following siRNA knockdown of DJ-1. B. Time-course of protein expression following cyclohexamide (CHX) treatment. Western blot analysis confirms the presence of Nrf2 at times following CHX treatment. C. *In cellulo* assay of Nrf2 ubiquitinylation. Nrf2 and covalently bound modifications were immuno-purified from Huh7 extracts and analyzed by SDS-PAGE Western blot analysis. D. Nrf2/KEAP1 co-immunoprecipitation. V5 epitope tagged KEAP1 was expressed in Huh7 cells with and without over expressed flag-DJ-1. Immunoprecipitation using anti-V5 antibody isolated endogenous Nrf2 protein and conversely, immunoprecipitation of endogenous Nrf2 isolated V5-KEAP1. H.C. denotes a cross-reacting band of IgG heavy chain present from the immunoprecipitating antibody.

Figure 4. Studies to determine how DJ-1 affects Nrf2-KEAP1 interaction. An antibody was used to purify KEAP1-containing protein complexes and then a semi-quantitative determination of how much Nrf2 was present in such complexes was carried out. Nrf2 is found in complex with KEAP1 under normal conditions in the cells (lane 1). However, overexpression of DJ-1 disrupts Nrf2-KEAP1 interaction (lane 2). The lysate blots are included to show that protein was loaded equally, and that the over expressed proteins were expressed equivalently.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations, and variations thereof.

As used herein, "a," "an" or "the" can mean one or more than one. For example, "a" cell can mean a single cell or a multiplicity of cells.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as an amount of dose (*e.g.*, an amount of a non-viral vector) and the like, is meant to encompass variations of ± 20%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

As used herein, the term "nucleic acid molecule" refers to a DNA or RNA molecule, including cDNA, a DNA fragment, genomic DNA, synthetic (e.g., chemically synthesized) DNA, plasmid DNA, mRNA, and anti-sense RNA, which can be single stranded or double stranded. Thus, nucleic acids of this invention can include a nucleic acid strand complementary to the described nucleic acid. A nucleic acid may or may not be immediately contiguous with nucleotide sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term includes, for example, a DNA molecule that is incorporated into a construct, into a vector, into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA that is part of a hybrid nucleic acid.

As used herein, the term "nucleic acid sequence" or "sequence" refers to the sequence of nucleotides from the 5' to 3' end of nucleic acid molecule. Nucleic acid sequences provided herein are represented using the standard code for representing the nucleotide characters as set forth in the U.S. sequence rules, 37 CFR §§1.821 - 1.825 and the World Intellectual Property Organization (WIPO) Standard ST.25.

As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA or antisense RNA. Genes may or may not be capable of being used to produce a functional protein. Genes include both protein-coding and non-coding regions (e.g., introns, regulatory elements, and 5' and 3' untranslated regions). A gene may be "isolated" by which is meant a nucleic acid that is substantially or essentially free from components normally found in association with the nucleic acid in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid. An "isolated" nucleic acid of the present invention is generally free of nucleic acid sequences that flank the nucleic acid of interest in the genomic DNA of the organism from which the nucleic acid was

derived (such as coding sequences present at the 5' or 3' ends). However, the nucleic acid of this invention can include some additional bases or moieties that do not deleteriously affect the basic characteristics of the nucleic acid.

As used herein, the term "fragment" or "active fragment thereof" or "domain" refers to a fragment or region (e.g., C terminal domain; N terminal domain; hydrophobic domain, etc.) of a protein or nucleic acid molecule that retains all or some of the activity of the original molecule. Methods for the generation of fragments and domains are known to those skilled in the art. For example, deletions can be done incrementally from the 3' end and/or the 5' end of a nucleotide sequence or from the carboxy terminus and/or amino terminus of an amino acid sequence. Deletions can also be made in the internal region of a nucleotide sequence or amino acid sequence to produce a fragment or domain of this invention. For each area of deletion, the size of the fragment to be deleted can be in an increment of, e.g., about 30 bp initially and about 10 bp later for further maximizing the size of deletion. The deletion mutants can be generated by using, for example, the Stratagene QuikChange Multi Site-Directed Mutagenesis kit. This method involves synthesis of mutant strands using primers containing desired mutations, digestion with DpnI to remove the parental plasmid, and transformation of the synthesized single-stranded plasmids into a bacterial host to be converted into double-stranded plasmids. Examples of fragments of the DJ-1 protein of this invention include but are not limited to any 10 contiguous amino acids of the amino acid sequence of the DJ-1 protein, as well as any multiples of 5 or 10 contiguous amino acids of the DJ-1 amino acid sequence (GenBank Accession No. NP 009193). Such fragments can be separate or combined in any combination of different fragments or the same fragments. Nonlimiting examples of a fragment of the DJ-1 protein to be included in this invention are amino acids 1-10, 1-20, 1-30, 1-40, 1-50, 1-100, 1-150, 1-189, 20-40, 50-70, 60-100, 70-120, 80-90, 100-189, 150, 189, etc., including any fragment identified by those amino acid numbers between 1 and 189 of the DJ-1 amino acid sequence not specifically recited herein. Fragments of this invention further include all DJ-1 nucleotide sequences that encode a DJ-1 fragment of this invention. Nucleic acid sequences for the DJ-1 protein of this invention are available, e.g., as GenBank Accession Nos. BC008188 and NM_007262.

As used herein, the term "cell" refers to any cell including cells in their native state in an organism, cells in a cell culture, and cells in a tissue or cell sample. The term cell as used herein is understood to encompass a single cell, multiple cells, and cells making up a specific tissue or organism.

The present invention relates to methods and compositions directed to the activity of DJ-1 as a modulator of Nrf2. Thus, in some embodiments, the present invention provides a method of identifying a gene involved in the oxidative stress response of a cell, comprising: a) contacting a cell with a molecule that reduces DJ-1 protein levels in a cell and thereby decreases the Nrf2 protein levels in the cell and then identifying a gene whose expression is altered as a result. Molecules for use in practicing the method of the present invention include but are not limited to siRNA molecules capable of reducing DJ-1 protein levels within the cell and thereby decreasing Nrf2 activity in the cell thus decreasing the cell's anti-oxidant response.

As used herein, the term "oxidative stress response" or "anti-oxidant response" refers to cellular responses to oxidative stress. There are many ways to assess a cell's anti-oxidant response. These include but are not limited to: measuring the expression of Nrf2 protein which accumulates in an anti-oxidant response; measuring the expression of Nrf2 regulated genes (e.g. NQO1, HO-1, GST, GCLM), whose activity is governed by their expression and depend on Nrf2 as a master regulator of function; measuring the oxidative state of cells, fluids, or tissues (this can be done quantitatively by various means including reduced fluorescent dyes and lumogenic compounds); measuring the degree of protection offered by a response to defined oxidative phenotypes (e.g., are the cells/tissues protected from cell death induced by oxidative stress [such as treatment with hydrogen peroxide, peroxy nitrite, nitric oxide, paraquat and others] or does the anti-oxidant response generated protect DNA from oxidative damage, and to what degree?); measuring the direct effect of oxidation on cellular macromolecules (this can be done by tracking endogenous proteins known to be directly oxidized (such as peroxiredoxins and DJ-1 itself) by methods such as 2-dimensional electrophoresis, or by assaying the oxidative state of lipids or even sugars); measuring the activation of the anti-oxidant response element (ARE) promoter DNA (this can be done in intact cells or cell-free *in vitro* transcription systems using cell lysates and can be measured either by the measurement of reporter genes under the regulation of the ARE promoter DNA, or by the measurement of natural endogenous genes with promoters that are under the control of the ARE (such as the NQO1 promoter)).

As used herein, "activity" includes but is not limited to protein production, protein function (e.g., binding activity, immunogenicity, gene regulating activity, etc.) gene expression to produce mRNA, translation of mRNA to produce protein, etc. DJ-1 activity includes DJ-1 protein production and stability in a cell, DJ-1 protein function (such as modulating Nrf2 activity, upregulating and/or downregulating gene expression, etc.), DJ-1

gene expression and mRNA stability and DJ-1 mRNA translation and post-translational modification. Nrf2 activity includes Nrf2 protein production and stability in a cell, Nrf2 protein function (such as interacting with DJ-1, acting as a transcription factor, binding KEAP1, etc.), Nrf2 gene expression and mRNA stability, and Nrf2 mRNA translation and post-translational modification.

As used herein, "modulate," "modulates" or "modulation" refers to enhancement (*e.g.*, increased activity and/or production) or inhibition (*e.g.*, diminished, reduced or suppressed activity and/or production) of the specified activity.

The term "enhancement," "enhance," "enhances," or "enhancing" refers to an increase in the specified parameter (*e.g.*, at least about a 1.1-fold, 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold or more increase) and/or an increase in the specified parameter of at least about 5%, 10%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95%, 97%, 98%, 99% or 100%. Methods useful for increasing the amount of DJ-1 or Nrf2 are known in the art and include but are not limited to using mRNA molecules, DNA transgenes, and protein delivery systems.

The term "inhibit," "diminish," "reduce" or "suppress" refers to a decrease in the specified parameter (*e.g.*, at least about a 1.1-fold, 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold or more increase) and/or a decrease or reduction in the specified parameter of at least about 5%, 10%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95%, 97%, 98%, 99% or 100%. In particular embodiments, the inhibition or reduction results in little or essentially no detectible activity (at most, an insignificant amount, *e.g.*, less than about 10% or about 5%). A substance that inhibits DJ-1 and/or Nrf2 activity according to the methods of this invention, can be, but is not limited to a ligand (e.g., an antibody or antibody fragment) that specifically binds a DJ-1 or Nrf2 protein or active fragment thereof and/or a nucleic acid that inhibits transcription or translation of nucleic acid encoding a DJ-1 and/or Nrf2 protein or active fragment thereof (e.g., an antisense nucleic acid that binds a coding sequence of the DJ-1 or Nrf2 protein, an interfering RNA that inhibits or suppresses transcription and/or translation of the DJ-1 or Nrf2 protein, a ribozyme, etc.) Furthermore, small molecules and other compounds and substances that inhibit the activity of DJ-1 and/or Nrf2 could be used in the methods of this invention.

Antisense therapy refers to administration or *in situ* generation of oligonucleotide probes or their derivatives which specifically hybridize or otherwise bind under cellular conditions with the cellular mRNA and/or genomic DNA encoding one of the polypeptides of the invention so as to inhibit expression of that polypeptide, e.g., by inhibiting transcription

and/or translation. This also relates to the use double stranded small interfering RNAs (siRNAs). RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing, particularly in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene (Elbashir et al. *Nature* 2001; 411(6836): 494-8). Accordingly, it is understood that siRNAs and long dsRNAs having substantial sequence identity to all or a portion of a polynucleotide of the DJ-1 can be used to inhibit the expression of a nucleic acid of DJ-1 or any chemically synthesized or plasmid based siRNA constructs against the DJ-1 target gene. This also should include any methods for administering siRNAs or antisense, such as introduction into the cell by physical methods, microinjection techniques, bombardment by particles, such as gene gun technology, electroporation, lipid-mediated, chemical-mediated, calcium phosphate or similar methods. In addition, any known gene therapy technique to administer RNA, such as a viral construct or expression construct that encodes transcription of siRNA is included herein.

As used herein, the term "siRNA molecule" refers to a small interfering RNA molecule capable of binding to a nucleotide sequence such as a messenger RNA (mRNA) molecule that shares a homologous sequence with the siRNA molecule. The use of siRNA employs a sequence-specific posttranscriptional gene silencing technique in which expression of the target gene is down regulated by introduction of homologous double-stranded RNA (dsRNA). An oligonucleotide is designed to contain a short sequence derived from target RNA separated by a spacer from the reverse complement of the same sequence. The resulting transcript folds back on itself, producing double-stranded siRNA. Selection of the target sequence can be performed with a computer program designed to identify mRNA sequences optimized for siRNA technique. SiRNA molecules useful for practicing the present invention include but are not limited to those provided as SEQ ID NO:1 and SEQ ID NO:2. Other siRNA molecules could be designed using the publicly available sequences for DJ-1 including DJ-1 DNA sequences available from NCBI as accession NM_007262 and BC008188 and the DJ-1 protein sequence available from NCBI as accession NP_009193. Additionally, any commercially available or publicly known siRNA sequence that down regulates DJ-1 could be used in practicing the present invention.

Also provided is a method of identifying a compound that has a modulating effect on the ability of DJ-1 to bind proteins with which it associates in a cell, under oxidative and non-oxidative conditions as described herein and or that are involved in the association of DJ-1 with Nrf2, including Nrf2, comprising: a) contacting the compound with DJ-1 and the protein under conditions whereby DJ-1 and the protein can bind; and b) determining the amount of

binding of DJ-1 and the protein, whereby an increase or decrease in the amount of binding of DJ-1 and the protein in the presence of the compound as compared to the amount of binding of DJ-1 and the protein in the absence of the compound identifies a compound that has a modulating effect on the ability of DJ-1 to bind the protein.

As used herein, the term "compound" refers to any protein or nucleic acid molecule, as well as any chemical substance consisting of chemically bonded chemical elements.

Additionally provided herein is a method of identifying a compound having the ability to modulate the production of DJ-1, comprising: a) contacting the compound with a cell that produces DJ-1; and b) determining the amount of DJ-1 mRNA and/or the amount of DJ-1 protein produced in the cell, whereby an increase or decrease in the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the presence of the compound as compared to the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the absence of the compound identifies a compound having the ability to modulate production of DJ-1.

The methods of this invention can be employed in *in vitro* assays, in single cells, in whole tissues, etc. DJ-1 is produced by cells, but modulating its production could be determined at the cellular, tissue, organ, or organism level. The amount of DJ-1 present in any tissue or fluid in a body can be determined. Examples of body tissues or fluids in which DJ-1 can be measured include but are not limited to any sample in which DJ-1 proteins and/or nucleic acids can be present. For example, the sample can be a body fluid, cells or tissue, including but not limited to, blood, serum, plasma, saliva, sputum, broncheoalveolar lavage, urine, semen, joint fluid, cerebrospinal fluid and cells, fluids and/or tissue from any organs in which DJ-1 can be detected, including lung, liver, heart, brain, kidney, spleen, muscle, etc.

In the screening methods of this invention, the source of the compound to be screened can be, but is not limited to, for example, a small molecule library, some of which are available commercially. Nonlimiting examples of libraries that can contain a compound of this invention include small molecule libraries obtained from various commercial entities, for example, SPECS and BioSPEC B.V. (Rijswijk, the Netherlands), Chembridge Corporation (San Diego, CA), Comgenex USA Inc., (Princeton, NJ), Maybridge Chemical Ltd. (Cornwall, UK), and Asinex (Moscow, Russia). One representative example is known as DIVERSet™, available from ChemBridge Corporation, 16981 Via Tazon, Suite G, San Diego, Calif. 92127. DIVERSet™ contains between 10,000 and 50,000 drug-like, hand-synthesized small molecules. The compounds are pre-selected to form a "universal" library that covers the maximum pharmacophore diversity with the minimum number of compounds

and is suitable for either high throughput or lower throughput screening. For descriptions of additional libraries, see, for example, Tan et al. "Stereoselective Synthesis of Over Two Million Compounds Having Structural Features Both Reminiscent of Natural Products and Compatible with Miniaturized Cell-Based Assays" *Am. Chem Soc.* 120, 8565-8566, 1998; Floyd et al. *Prog Med Chem* 36:91-168, 1999. Numerous libraries are commercially available, e.g., from AnalytiCon USA Inc., P.O. Box 5926, Kingwood, Tex. 77325; 3-Dimensional Pharmaceuticals, Inc., 665 Stockton Drive, Suite 104, Exton, Pa. 19341-1151; Tripos, Inc., 1699 Hanley Rd., St. Louis, Mo., 63144-2913, etc.

Because DJ-1 disrupts the interaction of Nrf2 and its inhibitor KEAP-1, the known DJ-1 crystallographic structure can be used to predict structures of pharmaceutics, biologics or mimetics, leading to the synthesis of such molecules which can be tested according to the methods provided herein for their ability to inhibit Nrf2 and KEAP1 interaction. Such molecules are expected to prevent the interaction of Nrf2 and KEAP1, thus prolonging the stability of Nrf2. Enhanced Nrf2 leads to enhanced anti-oxidant responses.

The effects of DJ-1 on inhibiting Nrf2-KEAP1 interaction, as well as on enhancing Nrf2 stability can be used as a basis for the development of assays to delineate or map domains and residues within DJ-1 that exert these functions. Once mapped, these domains can be used to design mimetics to identify small molecules that produce the same functions.

Further provided is a method of identifying a compound having the ability to modulate the activity of Nrf2, comprising: a) contacting the compound with a cell in which Nrf2 has activity; and b) determining the amount of Nrf2 activity in the cell, whereby an increase or decrease in the amount of Nrf2 activity in the cell in the presence of the compound as compared to the amount of Nrf2 activity in the cell in the absence of the compound identified a compound having the ability to modulate the activity of Nrf2. This method can be carried out in a cell, tissue, organ and/or organism.

The activity of DJ-1 or Nrf2 can be determined by: assaying the binding of DJ-1 to macromolecular binding partners; measuring the cell's anti-oxidant response; determining the affect of a given manipulation or alter defined phenotype endpoints linked with the function defined here (for example, DJ-1 protects cells from cell death; thus modified DJ-1 activity could be measured by the ability of a compound to protect cells from death as compared to normal DJ-1 activity); and assaying for known DJ-1 effects on Nrf2 protein (this includes but is not limited to Nrf2 protein expression, stability, and half-life; Nrf2 conjugation with ubiquitin; and physical association of Nrf2 with KEAP1).

As used herein, the terms "expression", "protein expression", or "gene expression" all refer to the process by which a gene's DNA sequence is used to produce an mRNA molecule that is subsequently used to produce a protein molecule. Expression can be measured by any of a variety of methods well known to one skilled in the art, including but not limited to, measurements of mRNA levels such as microarray analysis, quantitative PCR analysis, and Northern blot analysis, measurements of DNA levels such as Southern blot analysis and measurements of protein levels such as Western blot analysis, immunoprecipitation and any of a wide variety of known immunoassays.

Also provided is a method of identifying a compound that modulates an anti-oxidant response directed by DJ-1 or Nrf2 comprising: a) contacting the compound with DJ-1 and/or Nrf2 under conditions whereby an anti-oxidant response can occur; and b) determining the effect of the anti-oxidant response, whereby an altered (e.g., a decreased or increased amount or effect) anti-oxidant response in the presence of the compound as compared to the anti-oxidant response produced in the absence of the compound identifies a compound that modulates an anti-oxidant response directed by DJ-1 or Nrf2. Methods for assessing an anti-oxidant response are provided herein and include determining the amount or effect of an anti-oxidant response, whereby a decrease or increase in the amount and/or effect of the anti-oxidant response in the presence of a compound as compared to the amount and/or effect of the anti-oxidant response in the absence of a compound identifies a compound having the ability to regulate an anti-oxidant response directed by DJ-1 or Nrf2.

Further provided is a method for treating a disease by modulating an anti-oxidant response in a subject comprising administering an effective amount of a compound that modulates an anti-oxidant response directed by DJ-1 or Nrf2 to the subject. Diseases treatable by the methods of the present invention include but are not limited to neurodegenerative diseases such as Parkinson's disease, exposure to toxic agents, exposure to radiation poisoning, cancer, and diseases due to chronic inflammation, cardiovascular damage and fibrosis.

Also provided herein is a method of treating a disease or disorder in a subject, wherein the disease or disorder is associated with an altered anti-oxidant response, such as Parkinson's disease. Such a method comprises administering to a subject an effective amount of a compound as described herein. Such compounds include but are not limited to: oxidative species such as reactive oxygen (superoxide, hydrogen peroxide, hydroxyl radicals, etc.) and nitrogen species (e.g., peroxynitrite, nitric oxide, Deta-NO, etc.); anti-oxidant compounds such as beta-napthoflavone, or the food preservative tert-butyl hydroquinone and vitamin E;

organosulfur compounds such as diallyl sulfides; aromatic hydrocarbons, including many compounds classified as toxins (e.g., Dioxin); naturally occurring anti-oxidant compounds such as those found in non-dietary and dietary sources (e.g., those from cruciferous vegetables); and any combination thereof.

Also provided herein is a method of treating a disease or disorder in a subject, wherein the disease or disorder is associated with an altered anti-oxidant response, such as cancer. Such method comprises administering to a subject an effective amount of a compound of this invention. Such diseases may also benefit from a decreased anti-oxidant response augmented by inhibitors of this pathway such as the NQO1 inhibitors dicumarol and/or flavinoids. The present invention further provides a method of inhibiting DJ-1 activity in a cancer cell that produces DJ-1, comprising contacting the cancer cell with an SiRNA molecule of DJ-1 which can be an siRNA comprising the nucleotide sequences set forth in SEQ ID NO:1, in SEQ ID NO:2 and/or a combination of both.

A method is also provided herein of identifying a cancer cell having increased resistance to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to a non-cancer cell identifies a cancer cell having an increased resistance to a chemotherapeutic agent.

Further provided herein is a method of identifying a compound that decreases the amount of DJ-1 in a cancer cell in which DJ-1 is produced, comprising: a) contacting the compound with the cancer cell; and b) measuring the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell of (a), whereby a decrease in the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the presence of the compound as compared to the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the absence of the compound identifies a compound that decreases the amount of DJ-1 in the cancer cell in which DJ-1 is produced.

In addition, the present invention provides a method of identifying a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced, comprising: a) contacting the compound with the cancer cell; and b) determining the amount of DJ-1 activity in the cancer cell of (a), whereby an increase or decrease in the amount of DJ-1 activity in the cancer cell in the presence of the compound as compared to the amount of DJ-1 activity in the cancer cell in the absence of the compound identifies a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced.

A method is also provided herein, of identifying a cancer cell having increased resistance to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to the amount of DJ-1 and/or Nrf2 activity in a non-cancer cell identifies a cancer cell having an increased resistance to a chemotherapeutic agent. A chemotherapeutic agent that can be employed in the methods of this invention can be but is not limited to Taxol, paclitaxel, MEK kinase inhibitors and any combination thereof.

In further embodiments, the present invention provides a method of identifying a cancer cell that is susceptible to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to a non-cancer cell identifies a cancer cell that is susceptible to a chemotherapeutic agent. A chemotherapeutic agent can included but is not limited to mitomycin C, Vitamin K3, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1), 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone (MeDZQ) beta-lapachone and any combination thereof.

In yet additional embodiments, the present invention provides a method of treating a cancer associated with an altered oxidative response in a subject, comprising administering to the subject an effective amount of a compound that modulates DJ-1 activity, thereby modulating Nrf2 activity and treating the cancer associated with an altered oxidative stress response. A cancer of this invention can be but is not limited to, lung cancer, ovarian cancer, prostate cancer, breast cancer, colon cancer and leukemia.

In the treatment methods of this invention, the compound can be a small interfering RNA (siRNA), which can be, for example, a nucleic acid comprising, consisting essentially of and/or consisting of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof. The compound can also be an antibody, ligand or other compound that binds DJ-1, as well as any other compound that has a DJ-1 activity inhibiting effect.

The present invention further provides a method of identifying a chemotherapeutic agent having a therapeutic effect in a cancer cell, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent, whereby a decrease in the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent as compared to the amount of DJ-1 and/or Nrf2 activity in the absence of the chemotherapeutic agent identifies a chemotherapeutic agent having a therapeutic effect in the cancer cell.

Further provided is a method of identifying a chemotherapeutic agent that is resistant to Nrf2- mediated detoxification, comprising: a) contacting the chemotherapeutic agent with a cell under conditions whereby an Nrf2-mediated detoxification response can occur; and b) determining if the chemotherapeutic agent has a therapeutic effect on the cell, whereby a chemotherapeutic agent that has a therapeutic effect on the cell in the presence of the Nrf2-mediated detoxification response identifies a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification.

Additionally provided herein is a method of measuring the efficacy of a chemotherapeutic agent for treating a cancer in a subject, comprising: a) measuring the amount of DJ-1 activity in the subject before administering the chemotherapeutic agent to the subject; b) administering the chemotherapeutic agent to the subject; c) measuring the amount of DJ-1 activity in the subject during and/or after administering the chemotherapeutic agent to the subject; and d) comparing the amount of DJ-1 activity of (a) with the amount of DJ-1 activity of (c), whereby a decrease in the amount of DJ-1 activity of (c) identifies a chemotherapeutic agent having efficacy for treating the cancer in the subject.

As used herein, the term "chemotherapeutic agent" refers to a pharmacologic agent that is known to be of use in the treatment of cancer. Common chemotherapeutic agents are well known to those skilled in the art. A chemotherapeutic agent of this invention can be, but is not limited to, irinotecan, gemcytobine, cisplatin, carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfran, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide, tamoxifen, raloxifene, estrogen receptor binding agents, taxol, paclitaxol, gemcitabien, navelbine, famesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, floxuridine, mutamycin, vincristin, vinblastin, methotrexate, MEK kinase inhibitors, antibodies, small molecules (e.g., HERCEPTIN monclonal antibody, tyrosine kinase inhibitors, signal transduction inhibitors, etc.) as well as any analogue or derivative of a chemotherapeutic agent of this invention. Chemotherapeutic agents finding particular use in the practice of the present invention include but are not limited to mitomycin C, Vitamin K3, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (a.k.a. RH1), 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone (a.k.a. MeDZQ), beta-lapachone and any combination thereof. A chemotherapeutic agent of this invention can be present in a composition of this invention and/or employed in a method of this invention in any combination with other chemotherapeutic agents and/or other therapeutic agents. Dosage ranges for the

chemotherapeutic agents of this invention would be known and/or readily determined by one skilled in the art.

To measure the amount or level of DJ-1 or Nrf2 in cancer cells, expression of the protein and/or mRNA can be performed. The former includes but is not limited to ELISA, radio-immunoassays, and immunoblots. The latter includes but is not limited to reverse transcriptase-polymerase chain reaction (PCR), Northern RNA blots, real-time PCR, RNAse protection assays, etc.

In order to determine the effect of DJ-1/Nrf2 on chemotherapeutic treatment, any chemical/genetic manipulation that alters DJ-1 or Nrf2 (such as siRNA, dominant negative proteins, DJ-1/Nrf2 protein over expression, or oxidative/anti-oxidant treatment) can be carried out, along with chemotherapeutic treatment, and then a measurement of the effectiveness of cell killing (apoptosis/proliferation assays or tumor growth/size/regression *in vivo*) against control samples (e.g., with wild type DJ-1/Nrf2 expression/activity) can be determined.

The present invention additionally provides a method of identifying a cancer cell having decreased resistance to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby a decreased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to a non-cancer cell identifies a cancer cell having a decreased resistance to a chemotherapeutic agent.

NQO1 is a detoxification enzyme regulated by Nrf2, which is profoundly dependent on DJ-1. Other chemotherapeutic agents that are activated by other detoxification enzymes are included within the embodiments of this invention. Mitomycin C/K3 is known to be activated by NQO1. Therefore, DJ-1 expression (which affects NQO1 activity) is a good target for determining if a cancer will be sensitive to mitomycin C treatment.

The transcription factor Nrf2 controls the expression of several detoxification enzymes that can modify chemotherapeutic agents, causing them to lose efficacy. DJ-1 can act as a biomarker to identify tumors that would likely express such resistance enzymes. Therefore, these tumors could benefit from treatment that modifies Nrf2 activity or the activity of its downstream enzymes. Such Nrf2 inhibitory small molecules can be identified by the methods provided herein. Some transcriptional small Maf proteins inhibit Nrf2 mediated transcription, and represent such therapeutic targets.

Some anti-cancer compounds are activated by Nrf2 controlled enzymes. Nrf2 controls the reductase enzyme NQO1 and without DJ-1, NQO1 is expressed at much lower levels. NQO1 activates the anticancer compounds Mitomycin C and the synthetic vitamin K -

Menadione (also known as K3), causing them to become toxic. Thus, the present invention also provides a therapeutic approach in which DJ-1-overexpressing tumors would be sensitive to treatment regimens including these and similar drugs.

Thus, in some embodiments, the present invention provides methods of identifying compounds that control the Nrf2 anti-oxidant pathway and provides a different target (DJ-1) protein for screening.

Further provided is a method of identifying a chemotherapeutic agent having a therapeutic effect in a cancer cell, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent, whereby a decrease in the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent as compared to the amount of DJ-1 and/or Nrf2 activity in the absence of the chemotherapeutic agent identifies a chemotherapeutic agent having a therapeutic effect in the cancer cell.

Additionally provided is a method of identifying a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification, comprising: a) contacting the chemotherapeutic agent with a cell under conditions whereby an Nrf2-mediated detoxification response can occur; and b) determining if the chemotherapeutic agent has a therapeutic effect on the cell, whereby a chemotherapeutic agent that has a therapeutic effect on the cell in the presence of the Nrf2-mediated detoxification response identified a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification.

The methods of this invention can include compounds activated favorably by Nrf2/DJ-1/NQO1 activity such as mitomycin C, Vitamin K3, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1), 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone (MeDZQ) beta-lapachone and any combination thereof.

In additional embodiments, the present invention provides a method of downregulating the expression of a Nrf2-regulated gene that is involved in an anti-oxidant response in a cell comprising contacting the cell with an siRNA of DJ-1 as described herein, whereby the amount of DJ-1 in the cell is reduced, resulting in a reduction in the amount of Nrf2 in the cell and the downregulation of expression of the Nrf2-regulated gene.

The present invention also provides a method of upregulating the expression of an Nrf2-regulated gene that is involved in an anti-oxidant response in a cell, comprising contacting the cell with a nucleic acid molecule encoding a DJ-1 protein under conditions whereby the nucleic acid molecule can be expressed to produce DJ-1 protein, whereby the

amount of DJ-1 in the cell is increased, resulting in an increase in the amount of Nrf2 in the cell and upregulation of expression of the Nrf2-regulated gene.

Examples of Nrf2 regulated genes include but are not limited to NAD(P)H quinone oxidoreductase I (NQO1) [available from NCBI as GeneID:1728], heme oxygenase I (HO-I) [available from NCBI as GeneID:3162]; glucose 6 phosphate dehydrogenase (G6PD) [available from NCBI as GeneID:2539]; glutathione S-transferase (including 'P') (GST); gluathione cysteine ligase (including the modifier subunit) (GCL) [GCLM available from NCBI as GeneID:2730 and GCLC available from NCBI as GeneID:2729]; superoxide dismutase (including 2 & 3) (SOD) [SOD2 available from NCBI as GeneID:6648 and SOD3 available from NCBI as GeneID:6649]; glutathione S-reductase (GSR) [available from NCBI as GeneID: 2936], and any combination thereof.

The present invention further provides a method of downregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby downregulating expression of the gene. Nonlimiting examples of a gene the expression of which is regulated by DJ-1 activity in a cell is a translocase of outer mitochondrial membrane 20 gene, an RNA polymerase I transcription factor gene, a transmembrance EMP24 transport domain-containing protein gene, a lysosome-associated membrane protein 1 gene, an ATP-binding cassette, subfamily C, member 3 gene, a chloride channel, nucleotide sensitive, 1A gene, an oncogene DJ-1/PARK7 gene, a PC4- and SFRS1-interacting protein 1 gene, a leptin related gene/leptin receptor gene, a Ras-associated protein gene, a phosphatidylinositol glycan, Class B gene, an NAD(P)H dehydrogenase, quinone 1 gene, a HRAS-like suppressor 3 gene, a noggin homolog gene and any combination thereof.

Further provided herein is a method of upregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby upregulating expression of the gene. Nonlimiting examples of a gene the expression of which is upregulated by DJ-1 activity in a cell is a gremlin 1 homolog, cysteine knot superfamily gene, a calreticulin gene, a connective tissue growth factor gene and any combination thereof.

In additional embodiments, the present invention provides a method of identifying a subject as having a disease resulting from an altered cellular oxidative stress response, comprising: a) measuring the level of DJ-1 protein in the cell; and b) comparing the level of DJ-1 protein in the cell with the level of DJ-1 protein in a reference cell, whereby an altered

level of DJ-1 in the cell identifies a subject as having a disease resulting from an altered cellular oxidative stress response.

The present invention also provides a method of identifying a subject with a disease as having a poor prognosis resulting from an altered cellular oxidative stress response, comprising: a0 measuring the level of DJ-1 protein in the cell; and b) comparing the level of DJ-1 protein in the cell with the level of DJ-1 protein in a reference cell, whereby an altered level of DJ-1 in the cell identifies a subject with a disease as having a poor prognosis.

In addition, the present invention is directed to a method of identifying a compound that decreases the amount of DJ-1 in a cancer cell in which DJ-1 is produced, comprising: a) contacting the compound with the cancer cell; and b) determining the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell, whereby a decrease in the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the presence of the compound as compared to the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the absence of the compound identifies a compound that decreases the amount of DJ-1 in a cancer cell in which DJ-1 is produced.

Also provided herein is a method of identifying a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced, comprising: a) contacting the compound with the cancer cell; and b) determining the amount of DJ-1 activity in the cancer cell, whereby an increase or decrease in the amount of DJ-1 activity in the cancer cell in the presence of the compound as compared to the amount of DJ-1 activity in the cancer cell in the absence of the compound identifies a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced.

The present invention further provides a method of treating a cancer in a subject by inhibiting an anti-oxidant response of an Nrf2-regulated gene, comprising administering to the subject an effective amount of an siRNA of a DJ-1 protein as described herein, thereby reducing the amount of DJ-1 protein in the cell, resulting in a reduction in the amount of Nrf2 in the cell, the downregulation of expression of the Nrf2-regulated gene and an inhibition of the anti-oxidant response of the gene.

Further provided herein is a method of treating Parkinson's disease in a subject whose cells are deficient in DJ-1 and/or Nrf2 function by inducing expression of Nrf2-regulated genes, comprising administering to the subject an effective amount of a nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence can be expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

In addition, the present invention provides a method of protecting neurons from destruction in a subject with Parkinson's disease by inducing expression of Nrf2-regulated genes and wherein the cells of the subject are deficient in DJ-1 and/or Nrf2 function, comprising administering to the subject an effective amount of a nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence can be expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

In some embodiments, the present invention provides a method of identifying a compound that has a modulating effect on the ability of Nrf2 to bind KEAP1, comprising: a) contacting the compound with Nrf2 and KEAP1 under conditions whereby Nrf2 and KEAP1 can bind; and b) determining the amount of binding of Nrf2 and KEAP1, whereby an increase or decrease in the amount of binding of Nrf2 and KEAP1 in the presence of the compound as compared to the amount of binding of Nrf2 and KEAP1 in the absence of the compound identifies a compound that has a modulating effect on the ability of Nrf2 to bind KEAP1.

The methods for assessing the amount of protein binding or association used in the practice of the present invention include all *in vivo* and *in vitro* biochemical and biophysical assays for measuring protein association known to those skilled in the art. The methods include all biochemical and biophysical assays for measuring protein binding or association, including but not limited to protein association detected by co-precipitation, co-immunoprecipitation, yeast two-hybrid system, surface plasmon resonance (biacore) analysis, protein chip (the first protein or a peptide of that protein is placed on a solid-phase chip, while the other protein is tested for the ability to associate with the first protein), affinity column (using the first protein as an affinity bait for the second protein), and co-chromatography. Measurements include but are not limited to those performed using whole cell lysates, enriched/purified fractions of cell lysates, and/or enriched/purified recombinant proteins. Measurements may involve in cell and *in vitro* cell free studies.

For example, for the co-immunoprecipitation assay, protein association can be measured by co-immunoprecipitation in which an antibody to either Nrf2 or KEAP1 is used to isolate complexes containing that protein. The amount of the other protein in such complexes is then measured (e.g., if an antibody is used to isolate Nrf2 protein complexes, the amount of KEAP1 in those complexes can be measured by Western blot analysis).

However, other indirect methods can be used to assay Nrf2-KEAP1 association, and these include but are not limited to: staining cells/tissues for Nrf2 cellular localization (Nrf2 not bound to KEAP1 is found in the nucleus of cells, but when bound to KEAP1 is

maintained in the cytosol); measuring Nrf2 protein expression (KEAP1 functions to degrade Nrf2 protein; when Nrf2 is not bound to KEAP1, it is stabilized and accumulates in the cell); measuring Nrf2 DNA binding via methods including chromatin immunoprecipitation [ChIP] or gel shift analysis (Nrf2 when bound to KEAP1 is maintained inactive in the cytoplasm, so Nrf2 in the nucleus available to bind DNA is another measurement of Nrf2 not bound to KEAP1); measuring Nrf2 transcriptional activity by measuring the expression of Nrf2 regulated genes by mRNA or protein measurements (Nrf2 bound to KEAP1 is transcriptionally inactive, whereas free Nrf2 is able to induce the expression of many anti-oxidant enzymes); and measuring Nrf2-ubiquitin conjugation (KEAP1 functions to target Nrf2 for conjugation with the small protein ubiquitin; when Nrf2 is bound to KEAP1 it is rapidly ubiquitinylated, so measurement of Nrf2-Ub relies on Nrf2-KEAP1 interaction). These measurements can be done both in cells and *in vitro* cell-free studies.

The present invention further provides a method of identifying a compound having the ability to modulate the production of Nrf2, comprising: a) contacting the compound with a cell that produces Nrf2; and b) determining the amount of Nrf2 mRNA and/or the amount of Nrf2 protein produced in the cell, whereby an increase or decrease in the amount of Nrf2 mRNA and/or Nrf2 protein in the cell in the presence of the compound as compared to the amount of Nrf2 mRNA and/or Nrf2 protein in the cell in the absence of the compound identifies a compound having the ability to modulate production of Nrf2. This method can be carried out in a cell, tissue, organ and/or organism.

In addition, the present invention provides a method of identifying a domain on the DJ-1 protein that facilitates binding of DJ-1 to other proteins (e.g., Nrf2 and/or other proteins as described herein, including proteins that bind to DJ-1 under oxidative conditions), comprising: a) contacting a fragment comprising at least ten contiguous amino acids of the amino acid sequence of the DJ-1 protein with such other protein(s) (e.g., Nrf2) under conditions whereby binding can occur; and d) detecting binding of the fragment to protein, thereby identifying a domain on the DJ-1 protein that facilitates binding of DJ-1 to the protein.

In further embodiments, the present invention provides a method of identifying a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2, comprising: a) contacting a fragment comprising at least ten amino acids of the amino acid sequence of the DJ-1 protein with Nrf2 and KEAP1 under conditions whereby binding between Nrf2 and KEAP1 can occur; and d) determining the amount of Nrf2 bound to KEAP1 in the presence of the fragment as compared to the amount of Nrf2 and KEAP1 bound in the absence of the

fragment, whereby a decrease in the amount of Nrf2 bound to KEAP1 in the presence of the fragment as compared to the amount of Nrf2 bound to KEAP1 in the absence of the fragment identifies a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2.

The present invention further includes isolated polypeptides, peptides, proteins, fragments, domains and/or nucleic acid molecules that are substantially equivalent to those described for this invention. As used herein, "substantially equivalent" can refer both to nucleic acid and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an undesirable adverse functional dissimilarity between reference and subject sequences. In some embodiments, this invention can include substantially equivalent sequences that have an adverse functional dissimilarity. For purposes of the present invention, sequences having equivalent biological activity and equivalent expression characteristics are considered substantially equivalent.

The invention further provides homologs, as well as methods of obtaining homologs, of the polypeptides and/or fragments of this invention. As used herein, an amino acid sequence or protein is defined as a homolog of a polypeptide or fragment of the present invention if it shares significant homology to one of the polypeptides and/or fragments of the present invention. Significant homology means at least 30%, 40%, 50%, 60%, 65%, 75%, 80%, 85%, 90%, 95%, 98% and/or 100% homology with another amino acid sequence. Specifically, by using the nucleic acids disclosed herein as a probe or as primers, and techniques such as PCR amplification and colony/plaque hybridization, one skilled in the art can identify homologs of the polypeptides and/or fragments of this invention in any subject.

It is further contemplated that the present invention provides kits for detection of the polypeptides and/or fragments and/or antibodies of this invention in a sample. In one embodiment, the kit can comprise one or more antibodies of this invention, along with suitable buffers, wash solutions and/or other reagents for the detection of antibody/antigen complex formation. In an alternative embodiment, a kit of this invention can comprise a polypeptide, an antigenic peptide of the polypeptide of this invention, a fragment of this invention and/or an antigenic peptide of a fragment of this invention, along with suitable buffers, wash solutions and/or other reagents for the detection of antibody/antigen complex formation.

The present invention further provides a kit for the detection of nucleic acid encoding the polypeptides and/or fragments of this invention. For example, in one embodiment, the kit can comprise one or more nucleic acids of this invention, along with suitable buffers, wash

solutions and/or other reagents for the detection of hybridization complex formation and/or amplification product formation.

It would be well understood by one of ordinary skill in the art that the kits of this invention can comprise one or more containers and/or receptacles to hold the reagents (e.g., antibodies, antigens, nucleic acids) of the kit, along with appropriate buffers and/or wash solutions and directions for using the kit, as would be well known in the art. Such kits can further comprise adjuvants and/or other immunostimulatory or immunomodulating agents, as are well known in the art.

In further embodiments, the nucleic acids encoding the polypeptides and/or fragments of this invention can be part of a recombinant nucleic acid construct comprising any combination of restriction sites and/or functional elements as are well known in the art that facilitate molecular cloning and other recombinant DNA manipulations. Thus, the present invention further provides a recombinant nucleic acid construct comprising a nucleic acid encoding a polypeptide and/or biologically active fragment of this invention.

The present invention further provides a vector comprising a nucleic acid encoding a polypeptide and/or fragment of this invention. The vector can be an expression vector which contains all of the genetic components required for expression of the nucleic acid in cells into which the vector has been introduced, as are well known in the art. The expression vector can be a commercial expression vector or it can be constructed in the laboratory according to standard molecular biology protocols. The expression vector can comprise viral nucleic acid including, but not limited to, poxvirus, vaccinia virus, adenovirus, retrovirus and/or adeno-associated virus nucleic acid. The nucleic acid or vector of this invention can also be in a liposome or a delivery vehicle, which can be taken up by a cell via receptor-mediated or other type of endocytosis.

The nucleic acid of this invention can be in a cell, which can be a cell expressing the nucleic acid whereby a polypeptide and/or biologically active fragment of this invention is produced in the cell. In addition, the vector of this invention can be in a cell, which can be a cell expressing the nucleic acid of the vector whereby a polypeptide and/or biologically active fragment of this invention is produced in the cell. It is also contemplated that the nucleic acids and/or vectors of this invention can be present in a host animal (e.g., a transgenic animal), which expresses the nucleic acids of this invention and produces the polypeptides and/or fragments of this invention.

The nucleic acid encoding the polypeptide and/or fragment of this invention can be any nucleic acid that functionally encodes the polypeptides and/or fragments of this

invention. To functionally encode the polypeptides and/or fragments (i.e., allow the nucleic acids to be expressed), the nucleic acid of this invention can include, for example, expression control sequences, such as an origin of replication, a promoter, an enhancer and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites and transcriptional terminator sequences.

Nonlimiting examples of expression control sequences that can be present in a nucleic acid of this invention include promoters derived from metallothionine genes, actin genes, immunoglobulin genes, CMV, SV40, adenovirus, bovine papilloma virus, etc. A nucleic acid encoding a selected polypeptide and/or fragment can readily be determined based upon the genetic code for the amino acid sequence of the selected polypeptide and/or fragment and many nucleic acids will encode any selected polypeptide and/or fragment. Modifications in the nucleic acid sequence encoding the polypeptide and/or fragment are also contemplated. Modifications that can be useful are modifications to the sequences controlling expression of the polypeptide and/or fragment to make production of the polypeptide and/or fragment inducible or repressible as controlled by the appropriate inducer or repressor. Such methods are standard in the art. The nucleic acid of this invention can be generated by means standard in the art, such as by recombinant nucleic acid techniques and/or by synthetic nucleic acid synthesis or *in vitro* enzymatic synthesis.

The nucleic acids and/or vectors of this invention can be transferred into a host cell (e.g., a prokaryotic or eukaryotic cell) by well-known methods, which vary depending on the type of cell host. For example, calcium chloride transfection is commonly used for prokaryotic cells, whereas calcium phosphate treatment, transduction and/or electroporation can be used for other cell hosts.

As indicated above, the compositions (e.g., proteins, fragments, nucleic acids, inhibitory compounds, enhancing compounds) of this invention can be present in a composition comprising a pharmaceutically acceptable carrier. Thus, pharmaceutical compositions comprising a composition of this invention and a pharmaceutically acceptable carrier are also provided. The compositions described herein can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. See, e.g., Remington, The Science and Practice of Pharmacy (latest edition). In the manufacture of a pharmaceutical composition according to embodiments of the present invention, the composition of this invention is typically admixed with, inter alia, a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a carrier that is compatible with other ingredients in the pharmaceutical composition and that is not harmful

or deleterious to the subject. The carrier can be a solid or a liquid, or both, and is preferably formulated with the composition of this invention as a unit-dose formulation, for example, a tablet, which may contain from about 0.01 or 0.5% to about 95% or 99% by weight of the composition. The pharmaceutical compositions are prepared by any of the well-known techniques of pharmacy including, but not limited to, admixing the components, optionally including one or more accessory ingredients. In particular, it is intended that a pharmaceutically acceptable carrier be a sterile carrier that is formulated for administration to or delivery into a subject of this invention.

The pharmaceutical compositions of this invention include those suitable for oral, rectal, vaginal, topical, inhalation (e.g., via an aerosol) buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous, subconjunctival, intravesicular, intramuscular, intradermal, intraarticular, intrapleural, intratracheal, intraperitoneal, intracerebral, intraarterial, intracranial, intraocular, intratumoral, intravenous, etc.), topical (i.e., both skin and mucosal surfaces, including airway surfaces) and/or transdermal administration, although the most suitable route in any given case will depend, as is well known in the art, on such factors as the species, age, gender and overall condition of the subject, the nature and severity of the condition being treated and/or on the nature of the particular composition (i.e., dosage, formulation) that is being administered.

In particular embodiments, more than one administration (*e.g.*, two, three, four or more administrations) may be employed to achieve the desired level of nucleic acid expression.

In some embodiments of the invention, the compound or molecule is administered to the central nervous system (CNS). The vector can be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes. cortex, basal ganglia, hippocampus and amygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The compound or molecule can also be administered to different regions of the eye such as the retina, cornea, or optic nerve.

The nucleic acid or vector can be delivered into the cerebrospinal fluid (*e.g.*, by lumbar puncture) for more disperse administration. The compound or molecule can further be administered intravascularly to the CNS in situations in which the blood-brain barrier has been perturbed (*e.g.*, brain tumor or cerebral infarct).

The compound or molecule can be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intra-ocular, intracerebral, intraventricular, intranasal, intra-aural, intra-ocular (*e.g.*, intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.*, sub-Tenon's region) delivery.

Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tables, each containing a predetermined amount of the composition of this invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Oral delivery can be performed by complexing a composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers include plastic capsules or tablets, as known in the art. Such formulations are prepared by any suitable method of pharmacy, which includes the step of bringing into association the composition and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the composition with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet can be prepared by compressing or molding a powder or granules containing the composition, optionally with one or more accessory ingredients. Compressed tablets are prepared by compressing, in a suitable machine, the composition in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets are made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the composition of this invention in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions of this invention suitable for parenteral administration can comprise sterile aqueous and non-aqueous injection solutions of the composition of this invention, which preparations are preferably isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol,

vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The compositions can be presented in unit\dose or multi-dose containers, for example, in sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition of this invention in a unit dosage form in a sealed container can be provided. The composition can be provided in the form of a lyophilizate, which can be reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection into a subject. The unit dosage form can be from about 1 μg to about 10 grams of the composition of this invention. When the composition is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can be included in sufficient quantity to emulsify the composition in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit dose suppositories. These can be prepared by admixing the composition with one or more conventional solid carriers, such as for example, cocoa butter and then shaping the resulting mixture.

Pharmaceutical compositions of this invention suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that can be used include, but are not limited to, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof. In some embodiments, for example, topical delivery can be performed by mixing a pharmaceutical composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Pharmaceutical compositions suitable for transdermal administration can be in the form of discrete patches adapted to remain in intimate contact with the epidermis of the

subject for a prolonged period of time. Compositions suitable for transdermal administration can also be delivered by iontophoresis *(see,* for example, *Pharmaceutical Research* 3:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the composition of this invention. Suitable formulations can comprise citrate or bis\tris buffer (pH 6) or ethanol/water and can contain from 0.1 to 0.2M active ingredient.

Thus the present invention also provides a method of treating a cancer in a subject, comprising administering to the subject an effective amount of a composition of this invention to the subject, thereby treating the cancer in the subject. This can be done via single and/or sequential administration of the various compositions.

A subject of this invention is any subject that is susceptible to a disease of the present invention resulting from an altered cellular oxidative stress response and who may be in need of and/or who could acquire a beneficial effect from the treatment methods of this invention (e.g., a subject suspected of having or diagnosed with cancer or Parkinson's disease). The subject of this invention can be, for example, avian or mammalian and in some embodiments, is a human.

Efficacy of the treatment methods of this invention can be determined according to well known protocols for determining the outcome of a treatment. For example, in cancer treatment tumor size and quantity can be monitored to identify a decrease in the size and/or number of tumors, and/or assays of serum can be conducted to identify a decrease in the amount of cancer antigen present in the serum of a subject. Other determinants of efficacy of treatment, include, for example, overall survival, disease-free survival, time to progression and/or quality of life, as are well known in the art.

"Treat" or "treating" or "treatment" refers to any type of action that imparts a modulating effect, which, for example, can be a beneficial effect, to a subject afflicted with a disorder, disease or illness, including improvement in the condition of the subject (*e.g.,* in one or more symptoms), delay in the progression of the condition, prevention or delay of the onset of the disorder, and/or change in clinical parameters, disease or illness, etc., as would be well known in the art.

The neurodegenerative diseases of the present invention can be, but is not limited to, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, memory loss, etc. Other disorders that can be treated or prevented by the methods of this invention include, but are not limited to cardiovascular disorders (e.g., atherosclerosis), inflammation, radiation-induced damage, tissue damage and age-related disorders. The methods of this invention can also be employed in wound repair and memory enhancement.

The cancer of the present invention can be, but is not limited to, B cell lymphoma, T cell lymphoma, myeloma, leukemia, hematopoietic neoplasias, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkins lymphoma, Hodgkins lymphoma, uterine cancer, adenocarcinoma, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, bladder cancer, liver cancer, prostate cancer, ovarian cancer, primary or metastatic melanoma, squamous cell carcinoma, basal cell carcinoma, brain cancer, angiosarcoma, hemangiosarcoma, head and neck carcinoma, thyroid carcinoma, soft tissue sarcoma, bone sarcoma, testicular cancer, uterine cancer, cervical cancer, gastrointestinal cancer, and any other cancer now known or later identified (see, e.g., Rosenberg (1996) *Ann. Rev. Med.* 47:481-491, the entire contents of which are incorporated by reference herein).

"Effective amount" refers to an amount of a compound or composition that is sufficient to produce a desired effect, which can be a therapeutic effect. The effective amount will vary with the age, general condition of the subject, the severity of the condition being treated, the particular biologically active agent administered, the duration of the treatment, the nature of any concurrent treatment, the pharmaceutically acceptable carrier used, and like factors within the knowledge and expertise of those skilled in the art. As appropriate, an "effective amount" in any individual case can be determined by one of ordinary skill in the art by reference to the pertinent texts and literature and/or by using routine experimentation. (*See*, for example, Remington, *The Science And Practice of Pharmacy* (20th ed. 2000)).

In general, a dosage range from about 0.001μg/kg to about 500 mg/kg of a composition of this invention, including any dosage amount or dosage sub-range within this range, will have therapeutic efficacy, with all weights being calculated based upon the weight of the composition.

As another example, if the nucleic acid of this invention is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection, but can be as high as 10^{12}, 10^{15} and/or 10^{20} pfu per injection. In some embodiments, a subject can receive a single injection. If additional injections are necessary, they can be repeated at daily/weekly/monthly intervals for an indefinite period and/or until the efficacy of the treatment has been established. As set forth herein, the efficacy of treatment can be determined by evaluating the symptoms and clinical parameters described herein and/or by detecting a desired immunological response.

The exact amount of the nucleic acid or vector required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the

particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every nucleic acid or vector. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

The frequency of administration of a composition of this invention can be as frequent as necessary to impart the desired therapeutic effect. For example, the composition(s) can be administered one, two, three, four or more times per day, one, two, three, four or more times a week, one, two, three, four or more times a month, one, two, three or four times a year, etc., as necessary to control the condition. The different compositions described herein can be administered simultaneously and/or sequentially in any order, which can be repeated, reversed and/or otherwise varied. Intervals between sequential administrations of different compounds can be optimized according to methods known in the art such that an advantageously combined effect is achieved. The amount and frequency of administration of the composition(s) of this invention will vary depending on the particular condition being treated and the desired therapeutic effect.

The compositions of this invention can be administered to a cell of a subject *in vivo* or *ex vivo*. For administration to a cell of the subject *in vivo*, as well as for administration to the subject, the compositions of this invention can be administered, for example as noted above, orally, parenterally (e.g., intravenously or intra-arterially), by intramuscular injection, intradermally (e.g., by gene gun), by intraperitoneal injection, subcutaneous injection, transdermally, extracorporeally, topically, intratumorally or the like.

If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art while the compositions of this invention are introduced into the cells or tissues. For example, the nucleic acids and vectors of this invention can be introduced into cells via any gene transfer mechanism, such as, for example, virus-mediated gene delivery, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

EXAMPLES

DJ-1 promotes transcription of anti-oxidant genes

The DJ-1 protein was initially described as a protein that weakly transforms 3T3 cells when applied alone, but in the presence of the Ras proto-oncogene, DJ-1 enhances cellular transformation. DJ-1 was identified in a proteomic analysis of proteins that are altered in tumor cells upon treatment with Taxol and MEK inhibitor. The study also found that DJ-1 expression is elevated in cancer tissues but not in adjacent normal tissue. Further, over-expression of DJ-1 protects cells from apoptosis while the loss of DJ-1 leaves cells susceptible to cytotoxic killing. These all point to an anti-apoptotic function of DJ-1. Mutation of the DJ-1 gene that disrupts its expression is found by others to be linked to early onset Parkinson's disease. More recently, higher DJ-1 expression in breast cancer is found to be a poor prognostic marker of breast cancer. These studies underscore the importance of DJ-1, however the mechanism by which DJ-1 mediates its function remains a central issue that is poorly understood. The objective of this study is to explore the function of DJ-1. Small interference RNA (siRNA) was used to reduce DJ-1 expression in tumor cells, and cDNA profiling was performed to assess the consequence of abolishing DJ-1 expression. It was found that genes in the anti-oxidant response pathway are dependent on DJ-1. Nearly half of the genes identified contain an Nrf2 binding site in their promoter. The transcription factor Nrf2 is critically important for the control of many genes that protect cells from oxidative and toxic stress. Nrf2 and the genes it regulates have been implicated in both Parkinson's disease and cancer. DJ-1 appears to promote the function of Nrf2, and increased DJ-1 expression in cancer leads to a survival advantage of tumor cells by enhancing their anti-oxidant responses. In contrast, its mutation in Parkinson's disease subjects causes the demise of dopaminergic neurons.

Modulating the Level and Activity of DJ-1 in a Cell

An unbiased profiling approach using siRNA molecules capable of reducing DJ-1 protein levels was used to examine the effect of DJ-1 on global gene expression. Two different small interfering RNA (siRNA) molecules (the sequences of which are provided herein as SEQ ID NO:1 and SEQ ID NO:2) were used to knock down DJ-1 mRNA and protein expression in H157 non-small cell lung carcinoma cells. DJ-1 siRNA#1 (SEQ ID NO:1) caused a more modest reduction in DJ-1 expressed while siRNA#2 (SEQ ID NO:2) caused a more profound decrease (Figures 1A-C).

Identifying Genes Involved in the Oxidative Stress Response

Total RNA was then extracted from these cells and Affymetrix GeneChip® analysis (Affymetrix, Inc., Santa Clara CA) was used to determine the expression of over 25,000 gene transcripts in samples transfected with DJ-1 specific oligomers (2 samples), scrambled non-target oligomers (2 samples), or mock transfected cells without dsRNA (1 sample). The quality of the RNA from all samples was determined to be high based on formamide-agarose electrophoresis, and comparison of expression in 5' and 3' probe sets in housekeeping genes including glucose 3-phosphate dehydrogenase (G3PDH). Expression comparison data was done based on differences observed between both siDJ-1 arrays and all three negative control arrays. In order to ensure that changes warranting further study were stringently filtered, all expression differences less than three fold were excluded as well as all spots with a raw signal intensity of less than five-hundred units in the samples where the spot was determined to be present (P). This filtering produced a list of three genes that were increased in the absence of DJ-1 expression, and fourteen genes whose expression was decreased in cells lacking DJ-1 (Table 1).

TABLE 1

	Fold Change	Imperfect AP-1/ARE site	SEQ ID NO:
TRANSLOCASE OF OUTER MITOCHONDRIAL MEMBRANE 20	-5.02		
RNA POLYMERASE I TRANSCRIPTION FACTOR	-4.81	GGAGGCTGAGGCACGAGAAT	3
TRANSMEMBRANE EMP24 TRANSPORT DOMAIN-CONTAINING PROTEIN	-4.34	CGAGGCTGAGGCACGAGAAT	4
LYSOSOME-ASSOCIATED MEMBRANE PROTEIN 1	-4.33		
ATP-BINDING CASSETTE, SUBFAMILY C, MEMBER 3	-3.88	ACTCAATGACTCATCGGCCC	5
CHLORIDE CHANNEL, NUCLEOTIDE SENSITIVE, 1A	-3.79	TTGCGCTGAGTCAGTTCCTG	6
ONCOGENE DJ-1/PARK7	-3.69	CGGACGTGACGCAGCGTGAG	7
PC4- AND SFRS1-INTERACTING PROTEIN 1	-3.57		
LEPTIN RELATED GENE/LEPTIN RECEPTOR	-3.49		
RAS-ASSOCIATED PROTEIN	-3.38		
PHOSPHATIDYLINOSITOL GLYCAN, CLASS B	-3.35		
NAD(P)H DEHYDROGENASE, QUINONE 1	-3.32	TCACAGTGACTCAGCAGAAT	8
HRAS-LIKE SUPPRESSOR 3	-3.31		
NOGGIN HOMOLOG	-3.02		
GREMLIN 1 HOMOLOG, CYSTEINE KNOT SUPERFAMILY	+4.00		
CALRETICULIN	+3.27		
CONNECTIVE TISSUE GROWTH FACTOR	+3.10	GCATCCTGAGTCACACGCGT	9

Among the genes whose expression decreased in the absence of DJ-1, NAD(P)H Quinone Oxidoreductase 1 (NQO1) was identified. NQO1 activity has been implicated in the risk and prevention of cancer and neurodegenerative diseases. Of particular interest is the fact that NQO1 is regulated to a large degree by gene transcription, and furthermore, *nqo1* is a prototypic downstream effecter of the anti-oxidant transcription factor, Nrf2. The tf_search algorithm (C Kast, et al. *J Biol Chem* **278**:6787-94 (2003)) was used to search for putative

Nrf2 binding sites within 1,000 base pairs upstream of the transcriptional start site of the genes identified in expression profiling data. Seven out of seventeen genes that were changed by more than 3 fold contained putative Nrf2 binding sites upstream of their transcript (Table 1).

Real-time PCR analysis was then performed and siDJ-1#2 was found to reduce DJ-1 expression by over 80% (Figure 2D). The NQO1 gene was selected as a prototypic target gene of DJ-1. NQO1 expression was reduced to a similar extent, however, Nrf2 expression was not changed (Figure 2D). This indicates that the effect is gene specific, and if subsequent effects are found for Nrf2-dependent transcripts, the effect is not due to a reduction of Nrf2.

To further analyze the role of DJ-1 in the oxidative stress response of a cell, a reporter gene construct (pGL2-ARE) containing the firefly luciferase gene under control of the anti-oxidant response element (ARE) from the human NQO1 promoter was used to transform cells. Nrf2 binds this ARE sequence and drives the expression of the luciferase gene. Two liver cell lines, HepG2 and Huh7, were used for these experiments since previous studies have shown that Nrf2 activity can be induced in these cells by treatment with the non-toxic food preservative, tert-butylhydroquinone (tBHQ). DJ-1 expression was knocked down in these cell lines by siRNA#1 or siRNA#2 and the cells were transfected with the pGL2-ARE reporter construct. Flag-Nrf2 was transfected into cells as a positive control for luciferase activation. Cells were then treated with either 50 uM tBHQ or DMSO vehicle control for 18 hours, lysed, and luciferase expression was measured (Figure 2A). Over-expression of Nrf2 robustly activated luciferase expression driven by the ARE sites. Transfection with scrambled control siRNA (siCTL) produced a level of luciferase expression consistent with a basal level of Nrf2 activity, while treatment of this sample with tBHQ induced expression consistent with previous studies. In the presence of DJ-1 siRNA, not only was the luciferase reporter gene expressed at much lower levels, but expression was no longer stimulated by tBHQ treatment. This effect is specific for the ARE element, as siDJ-1 did not affect an SV40 promoter, the glucocorticoid responsive element, nor a cAMP responsive element (Figures 2B and 2C). Western blot analysis of the lysates showed that these considerable differences arise even when DJ-1 expression is knocked-down only moderately.

The expression of the NAD(P)H quinone oxidoreductase-1 (NQO1) gene was then further analyzed. NQO1 was selected as a prototypic target gene of DJ-1. Real-time Q-PCR analysis of mRNA expression following siRNA knockdown of DJ-1 was used to verify that NQO1 expression is decreased following DJ-1 knockdown. NQO1 transcription was

measured in DJ-1 deficient cells and found to be greatly decreased in the absence of DJ-1 (Figure 2D). However, Nrf2 is unaffected by the loss of DJ-1 at the mRNA level (Figure 2D).

Modulating the Level and Activity of Nrf2 in a Cell

Western blot analysis of lysates from cells containing DJ-1 siRNA revealed that Nrf2 protein expression was drastically reduced in the absence of DJ-1, with siRNA#1 causing a more modest decrease, and siRNA#2 causing a dramatic decreased (Figure 3A). This level of reduction is correlated with the reduction of DJ-1 by these two siRNAs, supporting the contention that the effect is due to the reduction of DJ-1.

To explore how DJ-1 caused a reduction of Nrf2 protein expression, DJ-1 expression was reduced in Huh7 cells using specific siRNAs, and then replicate cultures were treated with the translation inhibitor molecule, cyclohexamide (CHX) to prevent new protein synthesis. The cells were lysed at various points over a time course and the expression of Nrf2, DJ-1, and control actin was analyzed by Western blot. Nrf2 protein was drastically decreased in the absence of DJ-1, essentially disappearing by 90 minutes of CHX treatment. In cells transfected with either a control scrambled sequence or with transfection reagent alone, Nrf2 protein was stable over 90 minutes of CHX treatment. (Figure 3B). This indicates that optimal stability of Nrf2 is dependent on DJ-1.

Nrf2 activity is regulated to a large degree by its stability, which is tightly controlled by the association of Nrf2 with a cytosolic inhibitor protein, KEAP1. Under basal/uninduced conditions, Nrf2 remains bound to KEAP1, which targets Nrf2 for ubiquitination by a Cullin-3 dependent mechanism. Nrf2 is then degraded by the 26S proteosome, preventing its transcriptional activity. Given the data implicating DJ-1 in Nrf2 stability, the effect of DJ-1 on Nrf2 ubiquitination was examined with *in cellulo* ubiquitination assays for Nrf2 in Huh7 cells. (Figure 3C). Cells expressing epitope-tagged ubiquitin and Nrf2 were transfected with DJ-1 or pcDNA, and treated with a proteosome inhibitor. Nrf2 was immuno-precipitated from denatured lysates and the Ubiquitin-Nrf2 conjugates were determined by Western blotting for the ubiquitin epitope. Nrf2, which is constitutively ubiquitinated and degraded in the basal state, was ubiquitinated to a far lesser degree when DJ-1 was over-expressed. The addition of tBHQ resulted in little ubiquitinated Nrf2 and this is not affected significantly by DJ-1. The addition of the proteosome inhibitor, MG132, prevented degradation and allowed the visualization of more ubiquitinated Nrf2; however in the presence of DJ-1, there was still reduced ubiquitinated Nrf2.

Using a colony of DJ-1 knockout mice, along with wild-type littermate controls, primary untransformed mouse embryonic fibroblast (MEF) cell cultures were generated to study the effect of DJ-1 on Nrf2 across species and in untransformed/non-cancerous cells. Reporter gene constructs encoding the firefly luciferase gene under control of the human NQO1 promoter's Nrf2 binding sequence were used and these studies showed that without mouse-DJ-1, mouse-Nrf2 was unable to express normal levels of the reporter gene, both under basal conditions and following induction with the classical activator of Nrf2 transcription, tert-butylhydroquinone (tBHQ). This effect was specific since a similar luciferase reporter gene plasmid, under control of the unrelated viral SV40 promoter, was unaffected by DJ-1 expression.

The expression of genes in DJ-1 +/+ and DJ-1 -/- MEF cultures that are regulated by Nrf2 was also examined. These genes have been found to be inducible in wild type DJ-1 +/+ MEFs by tBHQ doses ranging from 25-100 μM. However, in cells lacking DJ-1, mouse NAD(P)H quinone oxidoreductase 1 (NQO1) and the mouse modifier subunit of glutathione cysteine ligase (mGCLM), did not induce to the levels of wild type MEFs, both differing from wild type induction by four (4) fold.

Using Western blot analysis, the effect of DJ-1 on Nrf2 protein expression was studied in the MEF cultures, similar to studies carried out in the human tumor cell-line cultures. Without DJ-1 expression, mouse Nrf2 protein did not accumulate following tBHQ treatment. By adding DJ-1 expression back to these DJ-1 -/- MEF cells, this effect was reversed, showing that the effect of DJ-1 was specific.

Having shown that restoring DJ-1 expression also restored Nrf2 protein expression, studies were carried out to demonstrate the effect of this restoration on the detoxification genes controlled by Nrf2. Adding DJ-1 back to DJ-1 deficient cells increased the expression of the Nrf2 regulated detoxification enzymes, mouse heme oxygenase 1 (mHmox-1), as well as mNQO1, even though it did not return them to completely normal levels.

The results of these studies indicate that DJ-1 affects mouse Nrf2 in primary mouse cells in the same way that it affects human Nrf2 in cancer cell-line models, which indicates that the DJ-1/Nrf2 axis is a conserved mechanism of detoxification present across species and seemingly unperturbed in disease states, specifically cancer. This indicates that the DJ-1/Nrf2 axis can be further studied in preclinical mouse models of cancer and that affecting Nrf2 is a primary function of the DJ-1 gene, not secondary to non-specific changes surrounding the methodology in human tumor cell-lines. Furthermore, the fact that reconstituting DJ-1 in knockout cell culture restores some Nrf2 function implies that DJ-1 is a

potential target for therapies in a wide variety of diseases involving oxidative stress. These include but are not limited to cancer, cardiovascular disease, neurodegenerative disorders (including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis), and aging-related disorders.

Examination of Protein-Protein Interaction Networks Involving DJ-1

Using recombinant DJ-1 (rDJ-1) protein generated in the inventors' lab, a protein array containing thousands of human recombinant protein targets was probed in a screening assay to determine putative protein targets that may directly interact with DJ-1. rDJ-1 was biotinylated and used to probe the protein microarray, positive interactions were detected using streptavidin-Alexa Fluor 647 fluorochrome conjugates, the array was scanned and then it was analyzed using GENEPIX PRO, PROTOARRAY PROSPECTOR, and JMP software. A positive interaction with rDJ-1 was defined as a fluorescence signal greater than two (2) standard deviations over the median fluorescence. This identified nine (9) positive interactions with DJ-1, of which three (3) were non-specific positives caused by errors in background fluorescence on the array (visually identified). Additionally, one (1) other positive protein interaction, Propionyl-CoA carboxylase (PCCA) encodes a biotin binding enzyme, which interacts with the biotin moieties of rDJ-1, not necessarily with DJ-1 itself. This leaves five (5) putative protein interactions that may bind DJ-1 protein directly including the following proteins: amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4 (ALS2CR4), silent mating type information regulation 2 homolog 7 (sirtuin 7 or Sirt7), WD repeat domain 5 (WDR5), plasminogen activator inhibitor 1 RNA binding protein (PAI1RBP; also known as PAI-1 mRNA binding protein and chromodomain helicase DNA binding protein3 interacting protein) and eukaryotic translation initiation factor 2, subunit 2 beta, 38 kDa (EIF2S2)

A separate approach was used to identify DJ-1 protein complexes. Using dual mass spectrometric analysis, proteins were identified by peptide sequence in DJ-1 containing complexes. In brief, human tumor cells were grown and protein lysates were extracted. DJ-1 containing protein complexes were immunopurified using the inventors' polyclonal anti-DJ-1 antibody and protein A-agarose resin. The resin-antibody-protein beads were washed extensively and proteins were isolated by organic elution. The proteins were then lyophilized, reconstituted, and enzymatically digested with trypsin. The peptide solution was analyzed by q-Tof LC/MS/MS mass spectrometric analysis and peptide sequences were analyzed against a database of human protein tryptic fragments, identifying the proteins

bound to DJ-1. These data were then filtered to exclude immunoglobulin peptides and contaminating cytoskeletal proteins. Surprisingly, immunoprecipitation of either DJ-1 or a control protein that isn't expressed in these cells yielded no specific interactors. However, when DJ-1 containing protein complexes were isolated from cells exposed to hydrogen peroxide at levels known to oxidize DJ-1 protein, twenty (20) putative protein binding partners of DJ-1 were identified, including: Proapolipoprotein A1; haptoglobin Hp2; lipoprotein CIII; alpha-1-antitrypsin (aa 268-394); amyloid fibril protein=transthyretin-related; glyceraldehyde-3-phosphate dehydrogenase; ADP/ADT translocator protein; Chain, Human Serum Albumin In A Complex With Myristic Acid And Tri-Iodobenzoic Acid; ATP-binding cassette, sub-family A member 3; complement component 3 precursor; Hypothetical protein MGC20781; transferrin; heat shock 70kDa protein 8 isoform 1; hypothetical protein LOC345651; ADP/ATP carrier protein; and carbamyl phosphate synthetase. This is consistent with a model where the majority of DJ-1 remains unbound to other proteins until cells are exposed to oxidative stress. When DJ-1 itself is oxidized, it can then complex with many other proteins, thereby exerting a function in response to oxidative stress. Oxidative stress is the major activating stimulus of Nrf2.

Given the data suggesting that DJ-1 may remain largely unbound in the absence of oxidative stress, gel filtration was used to determine the molecular size of DJ-1-containing complexes under varying oxidative conditions. Tumor cell lysates exposed to oxidative conditions or control media were fractionated using fast protein liquid chromatography (FPLC) through gel filtration columns that isolate large (>100 kDa) or small (<100 kDa) protein complexes. Assays for DJ-1 were carried out using Western blot analysis on the various elution fractions. As the mass spectrometric data suggested, the majority of unoxidized DJ-1 was found not t be present in large protein complexes, but instead elutes from the column at retention times consistent with monomeric or dimeric DJ-1. However, when oxidizing stimuli are present, DJ-1 binds to proteins forming a larger complex. Determining the size of such DJ-1 containing complexes and identifying the proteins present in each of the discreet DJ-1 containing complexes will allow for a definition of specific functional units of DJ-1 activity following oxidative stress, and preceding Nrf2 activation and/or cellular survival or apoptosis signaling. Furthermore, these DJ-1 functional units present potential targets for therapeutic intervention in diseases with an oxidative component, including but not limited to cancer, cardiovascular disease (e.g., atherosclerosis), neurodegenerative disorders (including Parkinsonism, Alzheimer's disease, amyotrophic lateral sclerosis), and aging-related disorders.

Development of DJ-1 Quantitation Methods Suitable for Clinical Use

DJ-1 is known to be a potential tumor biomarker. DJ-1 is present at detectable levels both in serum and tissue biopsies. A high throughput method for quantifying DJ-1 protein has been developed. This method uses a direct enzyme linked immunoadsorbent assay ("sandwich" ELISA), to quantify DJ-1 protein, even at low picogram levels. This method uses microtiter plates bound with the inventors' rabbit polyclonal anti-DJ-1 antibody to capture DJ-1 protein present in solutions including human serum and non-denaturing cell lysates. The bound DJ-1 is then detected using a commercially available monoclonal mouse IgG raised against DJ-1 (Stressgen) that has been biotinylated, followed by neutravidin-peroxidase . Peroxidase activity, and therefore DJ-1 quantity, is measured by the colorigenic breakdown of the peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB). This method allows for the precise quantification of recombinant DJ-1 protein standards with a correlation coefficient of 0.997.

Using this method to quantify DJ-1 levels in human serum, a pilot study has been initiated to measure serum DJ-1 in eight (8) patients with diagnosed head or neck cancer and in five (5) healthy control patients. This study was intended to determine if DJ-1 levels could be reliably measured in human serum as a proof of principle. DJ-1 serum levels were detected and precisely quantified in all patients tested. Additionally, one of the cancer patients had significantly increased serum DJ-1. This pilot study shows that this method of DJ-1 quantification is powerful enough to detect differences in DJ-1 serum expression within a group of patients (Table 2). Patients are labeled in an arbitrary order where 'C' indicates patients diagnosed with head or neck cancer and 'N' indicates normal healthy volunteers. A cell lysate sample was included as a positive control for DJ-1 expression.

TABLE 2

Sample:	Mean Concentration (pg/mL)	Standard Error
C1	5	0.40
C2	5	0.69
C3	2	0.40
C4	5	0.69
C5	5	0.80
C6	15	4.33
C7	99	22.15

C8	11	1.06
N2	15	9.01
N3	6	0.40
N4	4	0.80
N6	15	0.80
N7	18	12.50
Lysate	201	

DJ-1 regulation of Nrf2 activity in Parkinson's disease

SH-SY5Y neuronal cells from the American Type Culture Collection (ATCC®) can be used for analysis of DJ-1 regulation of Nrf2 activity in Parkinson's disease. This cell line is derived from a metastatic neuroblastoma tumor and displays many of the phenotypic characteristics of normal dopaminergic neurons. These cells can be maintained in culture according to ATCC® protocol.

The quinone electrophile tert-butyl hydroquinone (tBHQ) is a non-toxic chemical preservative in food and other products. In addition to its chemical preservative properties, tBHQ potently induces Nrf2 activation and transcription at pharmacological/micromolar concentrations. For analysis, tBHQ can be administered dissolved in dimethyl sulfoxide (DMSO). In all cases where tBHQ is used, a DMSO only control can be included to separate any effects of the solvent vehicle from those of the drug. In cell culture, the DMSO final concentration would be less than 0.01% in normal culture media.

Using directional PCR cloning, mammalian expression plasmids were created encoding the open reading frame of the NQO1 cDNA and the KEAP1 cDNA, both in frame with 3' terminal V5 and His epitope tags, named pcDNA3.1D-NQO1 and pcDNA3.1D-KEAP1, respectively. When transfected into cells, these plasmids can express epitope tagged protein at high levels. A Flag-tagged Nrf2 construct (pcDNA-Flag-Nrf2) and a Flag-tagged DJ-1 (pCMV-Flag-DJ-1) can be used and have been previously described (Y Hod, et al. (1999) *J Cell Biochem* 72:435; M Furukawa, et al. (2005) *Mol Cell Biol* 25:162). Plasmid DNA can be transfected into cells using FuGene™6 (Roche Diagnostics Corporation, Indianapolis IN) according to the manufacturer's protocol. Knockdown of DJ-1 expression can be achieved by any method known to one skilled in the art. Exemplary methods include transfection of double stranded siRNA oligomers into cells using Oligofectamine™ (Invitrogen Corporation, Carlsbad CA) according to the manufacturer's protocol. Other

methods include: alternate transfection lipids such as Lipofectamine™ 2000 (Invitrogen Corporation, Carlsbad CA) and TransPass™ (New England Biolabs, Ipswich MA), as well as Amaxa Nucleofector® systems (Amaxa Biosystems, Cologne, Germany). Controls may be included and DJ-1 knockdown can be monitored by Western blot analysis of protein expression using a high titer DJ-1 antibody.

Immunoprecipitation of protein complexes can be performed using Immobilized Protein A/G Agarose (Pierce Biotechnology, Inc., Rockford IL) and antibodies capable of immunoprecipitation such as Anti-V5 Antibody (Invitrogen Corporation, Carlsbad CA), anti-Nrf2 (H-300) Antibody (Santa Cruz Biotechnology, Santa Cruz CA), and ANTI-FLAG® M2 Antibody (Sigma-Aldrich Co., St. Louis MO). Immunoprecipitation can be done with 1% Triton® X-100 (Sigma-Aldrich Co., St. Louis MO) as lysis, binding, and wash buffer. Agarose samples can be boiled in a denaturing detergent buffer with added reducing equivalents to elute the antigen complexes, which can then be analyzed.

In the absence of DJ-1, NQO1 is no longer transcribed. Additionally, transcription regulated by the anti-oxidant response element (ARE) is abrogated in cells lacking DJ-1 expression. Furthermore, this deficit is not overcome by treatment with classical stimuli of the factor largely responsible for ARE transcription, Nrf2. While the mRNA transcript for Nrf2 is not altered following DJ-1 knock-down, Nrf2 protein is expressed at a significantly lower level in cells deficient in DJ-1. DJ-1 may be affecting Nrf2 protein stability. Nrf2 binds to the cytosolic inhibitory protein, KEAP1. KEAP1 prevents Nrf2 transcription by sequestering Nrf2 in the cytoplasm and targeting Nrf2 for proteosome dependent protein degradation. DJ-1 may stabilize Nrf2 protein by preventing KEAP1 mediated protein degradation.

The effect of DJ-1 on the stability of Nrf2 protein can be determined using a pulse-chase experimental approach. SH-SY5Y cells would be cultured and transfected with siRNA oligomers to knockdown DJ-1 expression as described above. Following siRNA transfection (48-72 hours later), the cells would be transferred to a methionine free culture medium, and subsequently pulsed with ^{35}S-methionine for a short time (~10 minutes). This would label proteins synthesized at the time of the pulse with radioactive methionine residues. Cell samples would then be washed and lysed at various times beginning immediately after the ^{35}S pulse, up to and including 24 hours later. Nrf2 protein would then be immunoprecipitated from the lysates as described above. Immunoprecipitated protein eluates would be separated by molecular weight using SDS polyacrylimide gel electrophoresis (SDS-PAGE). The gels would be dried, and developed on X-ray film using a phosphoimager screen. Subsequently,

to determine whether Nrf2 degradation depends on the activity of the 26S proteosome, the pulse-chase experiment would be repeated in parallel using samples treated with the proteosome inhibitor, MG132.

In order to minimize complications caused by the fact that active/stable Nrf2 accumulates preferentially in the nucleus of cells, lysis conditions would be used that ensure nuclear lysis. This may affect the ability of the H-300 antibody to immunoprecipitate Nrf2, since such lysis conditions often denature proteins and the H-300 IP epitope recognition may require secondary protein structure. Nrf2 containing the Flag(R) epitope tag would be alternately exogenously expressed to account for this. This would allow SDS-based nuclear lysis and later dilution to non-denaturing antibody binding conditions since the immunoprecipitated antibody binds primary protein structure.

To further clarify the specific role of DJ-1 in Nrf2 protein stability, the pulse chase experiment would be repeated with over expressing DJ-1 at greater than wild type levels using a mammalian expression plasmid encoding DJ-1. The half-life of Nrf2 protein would be expected to be greater in cells with over expressed DJ-1 than in wild type cells.

The role of DJ-1 on Nrf2 interaction with its cytosolic regulator protein, KEAP1

In the absence of stimuli that induce Nrf2 mediated transcription, KEAP1, the cytosolic inhibitor of Nrf2, recruits Cullin-3/Roc-1 ubiquitination machinery targeting the Nrf2 protein for degradation. When cells are exposed to oxidant or toxic stimuli, Nrf2 dissociates from KEAP1. This physical dissociation of Nrf2 from KEAP1 leads to the activation, stabilization, and accumulation of Nrf2 in the nucleus. This mechanism of Nrf2 stabilization and activation plays a key role in the regulation of Nrf2 function. Since loss of DJ-1 leads to loss of Nrf2, DJ-1 could stabilize Nrf2 protein by disrupting binding to KEAP1. This can be examined by looking at the effects of DJ-1 on KEAP1/Nrf2 association.

KEAP1/Nrf2 binding in cells would be measured by using semiquantitative co-immunoprecipitation. These experiments would be done using a DJ-1 over-expression system. The specific effect of DJ-1 over-expression on Nrf2/KEAP1 interaction would be determined. This approach would be necessary given that loss of DJ-1 leads to decreases in Nrf2 protein levels. A siRNA knockdown of DJ-1 expression would decrease Nrf2, which would complicate the measurement of KEAP1/Nrf2 interaction.

SH-SY5Y cells would be transfected using either pCMV-Flag-DJ-1, or empty vector control DNA. Cell samples would then be treated with vehicle control or with tert-butylhydroquinone (tBHQ). Treatment with tBHQ would trigger Nrf2 activation and

dissociation from KEAP1 in wild type cells. Following drug treatment, the cells would be washed and lysed. Endogenous Nrf2 protein would then be immunoprecipitated. The immunoprecipitated eluates for KEAP1 content would be analyzed by Western blotting with antibodies that bind the KEAP1. The intensity of the bands on Western blot would represent a semiquantitative measurement of the KEAP1 protein that is bound to Nrf2.

These results would be verified by repeating the experiment and reversing the antibodies used for immunoprecipitation and Western blotting. KEAP1 complexes would be isolated from the cell lysates and the amount of endogenous Nrf2 bound in those complexes would be measured.

Role of Nrf2 in DJ-1 deficient neuronal cell death in vitro

Much of the understanding of Parkinson's disease stems from animal models that use chemicals to approximate the human disease. Originally identified as a contaminant in illicit drugs, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a disorder similar to Parkinson's disease by killing the same dopaminergic neurons that are affected in Parkinson's disease. In humans and higher primates, MPTP is converted to a metabolite, the methylpyridinium ion (MPP+), which is highly toxic to the neuron.

Similarly, the compound 6-hydroxydopamine (6-OHDA) has also been used to model Parkinson's disease. Dopamine is oxidized under physiological conditions to form quinone species. Dopamine quinones are highly reactive, and can oxidize protein residues leading to toxic effects on neurons. The spontaneous 1,4 addition of water to dopamine quinone produces 6-OHDA, which kills dopaminergic neurons both *in vivo* and *in vitro*.

Treatment with either MPP^+, or 6-OHDA induces apoptosis of SH-SY5Y cells. DJ-1 expression has been knocked down by siRNA and used to show that SH-SY5Y cells were more susceptible to killing by MPP+ and 6-OHDA in the absence of DJ-1 expression. Treatment of SH-SY5Y cells with tert-butylhydroquinone (tBHQ) protects SH-SY5Y cells from apoptosis following the same treatments. Linking these two is the association of DJ-1 with the regulation of Nrf2. Loss of Nrf2 may therefore be caused by the lack of functional DJ-1 in early onset Parkinson's disease, contributing to the loss of dopaminergic neurons. Restoring the expression of Nrf2 and its downstream target NQO1 to cells lacking DJ-1 may bypass the DJ-1 deficiency and protect SH-SY5Y neurons from apoptotic cell death.

To test this, SH-SY5Y cells grown in culture would be transfected using siRNA oligomers to knockdown DJ-1 expression. Following siRNA knockdown (36-48 hours later)

cells would be transfected with plasmid DNA containing either the gene for Nrf2 or NQO1. The next day, the cells would be treated with varying concentrations of the model cytotoxic compounds, MPP+ or 6-OHDA. Cell viability and apoptotic cell death would be assayed 12 hours later. This time course would coincide with near maximal knockdown of DJ-1 protein levels at the time of drug treatment and correlate with previously reported studies allowing a direct comparison of results.

Cell viability and death would be assayed using three different biological assays to conclude that cell killing or protection from killing involves a particular cellular pathway. This would allow more precise understanding of the mechanism of DJ-1 dependent cellular protection. Cell viability would be assayed using the cleavage of the soluble tetrazolium salt, XTT (Roche Diagnostics Corporation, Indianapolis IN). This assay would measure cellular metabolism of live cells and directly correlate with viability. Apoptotic and non-apoptotic cell death would be differentiated by staining the cells with Annexin-V (BD Biosciences, San Jose, CA) and propidium iodide. These two assays would measure cell membrane asymmetry (apoptosis) and membrane integrity (necrosis), respectively. Another assay for apoptotic cell killing using the Apo-ONE® assay (Promega Corporation, Madison WI) would also be done. This would utilize a profluorescent caspase 3/7 substrate that when cleaved would produce an active rhodamine fluorochrome. By measuring the fluorescent intensity produced by cellular lysates the intact caspase 3/7 activity, which is a specific indicator of apoptotic cell death, would be measured.

The effect of DJ-1 on Nrf2 function in vivo in a mouse model.

The brain is a complex organ with many emergent properties. Therefore laboratory animal models are used to confirm the biological relevance of molecular and cellular findings. Mice have been genetically engineered that lack the DJ-1 gene. Unfortunately, these mice do not provide an adequate Parkinson's disease model, mimicking all of the symptoms of the disease; instead they exhibit a relatively mild movement disorder. However, it is unknown how environmental toxic insults might affect these mice. These DJ-1 knockout (KO) mice provide a unique and powerful tool to study the effects of DJ-1 on Nrf2/NQO1 *in vivo*. Mice lacking DJ-1 may have disrupted Nrf2 function, leading to diminished expression of the detoxification enzyme NQO1.

To test this, mice lacking the DJ-1 gene which were generated in the laboratory of Tak Mak at the University of Toronto (Toronto, CA) would be used. The effect of DJ-1 on Nrf2 in mice would be verified by quantitatively determining the expression of Nrf2 protein

and several Nrf2 regulated gene transcripts in the mice. These values would be measured both in the brain, where DJ-1 may affect dopaminergic cell death, and in the liver, where Nrf2 mediated detoxification plays an important biological role. The baseline expression 6-week old female and male mice, as well as 12-week old female and male mice would be determined. At 12 weeks of age, the mice lacking DJ-1 exhibit decreased movement compared to wild type mice; this suggests that pathological changes are present at this age. This difference is not observed in 6-week old animals. The brain and liver of the mice from each sample set would be lysed and both protein and total RNA would be isolated. The total RNA would then be quantified, DNAse treated to remove any contaminating DNA, and then reverse transcribed to produce cDNA.

Protein samples from the mice would be separated by SDS-PAGE and the expression of Nrf2 would be determined by Western blot analysis. Nrf2 protein expression would be assayed using anti-Nrf2 (H-300) Antibody (Santa Cruz Biotechnology, Santa Cruz CA) that cross reacts with both human and mouse Nrf2.

Mice liver and brain cDNA samples would be analyzed using quantitative real-time PCR. The expression of the control RNA transcripts,18S ribosomal RNA (rRNA) and GAPDH (mRNA), would be measured. The Nrf2 regulated genes NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HO-1), epoxide hydrolase 1 (Ephx-1), and glutathione cysteine ligase modifier subunit (GCLM) would also be measured. The expression of these four genes is regulated in part or in whole by the transcriptional activity of Nrf2.

The ability of these mice to mount a Nrf2 mediated response to the well known Nrf2 activating compound tBHQ would then be tested. Both knockout and wild type mice would be challenged with tBHQ or vehicle control (DMSO) by intraperitoneal injection. This is a standard administration route. The liver and the brain of these animals would then be harvested and assayed for Nrf2 protein and NQO1, HO-1, Ephx-1, and GCLM mRNA expression.

In all the experiments using DJ-1 KO mice, wild type control mice from the parental strain would be included in order to alleviate DJ-1 independent strain effects. Additionally, the DJ-1 KO mice would be backcrossed for at least 10 generations onto the parental strain to ensure that results would not be complicated by any non-linked genetic differences in the mice. Wild type cDNA samples would be included in every real-time q-PCR reaction since these samples express DJ-1. They will act as a positive control for the PCR amplification. Conversely, non-template control samples that do not contain cDNA would be included as

well. Any gene expression measured in these samples would represent contamination of the reaction mixture.

DJ-1 is expected to affect Nrf2 in mice as it does in human cell culture. However, if it does not it could offer an explanation as to the differences seen between the human mutation that leads to Parkinson's disease and the relatively mild knockout mouse phenotype. The DJ-1 knockout mice would then be crossed with Nrf2 knockout mice to evaluate the more complex phenotype.

Except as otherwise indicated, standard methods known to those skilled in the art may be used for the construction and use of nucleic acid molecules, vectors, selectable markers, cells, transgenic organisms, and the like. Such techniques are well known to those skilled in the art. See, e.g., J. Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3rd Ed. (2001) (Cold Spring Harbor Laboratory Press; Woodbury, NY); *Current Protocols In Molecular Biology*, edited by F. M. Ausubel et al. (John Wiley & Sons, Inc.; Hoboken, NJ); and *Current Protocols in Cell Biology*, edited by Juan S. Bonifacino, et al. (John Wiley & Sons, Inc.; Hoboken, NJ).

All publications, patents and patent publications cited herein are incorporated by reference herein in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

CLAIMS

What is claimed is:

1. A method of identifying a compound having the ability to modulate an anti-oxidant response directed by DJ-1 and/or Nrf2, comprising:

a) contacting the compound with DJ-1 and/or Nrf2 under conditions whereby an anti-oxidant response can occur; and

b) determining the amount or effect of the anti-oxidant response, whereby a decrease or increase in the amount and/or effect of the anti-oxidant response in the presence of the compound as compared to the amount and/or effect of the anti-oxidant response in the absence of the compound identifies a compound having the ability to modulate an anti-oxidant response directed by DJ-1 or Nrf2.

2. A method of identifying a compound having the ability to modulate the production of DJ-1, comprising:

a) contacting the compound with a cell that produces DJ-1; and

b) determining the amount of DJ-1 mRNA and/or the amount of DJ-1 protein produced in the cell, whereby an increase or decrease in the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the presence of the compound as compared to the amount of DJ-1 mRNA and/or DJ-1 protein in the cell in the absence of the compound identifies a compound having the ability to modulate production of DJ-1.

3. A method of identifying a compound having the ability to modulate the activity of DJ-1, comprising:

a) contacting the compound with a cell in which DJ-1 has activity; and

b) determining the amount of DJ-1 activity in the cell, whereby an increase or decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the activity of DJ-1.

4. A method of identifying a compound having the ability to modulate the production of Nrf2 by modulating DJ-1 activity, comprising:

a) contacting the compound with a cell in which DJ-1 has activity; and

b) determining the amount of DJ-1 activity in the cell, whereby an increase of decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the production of Nrf2 by modulating DJ-1 activity.

5. A method of identifying a compound having the ability to modulate the activity of Nrf2 by modulating DJ-1 activity, comprising:

a) contacting the compound with a cell in which DJ-1 has activity; and

b) determining the amount of DJ-1 activity in the cell, whereby an increase or decrease in the amount of DJ-1 activity in the cell in the presence of the compound as compared to the amount of DJ-1 activity in the cell in the absence of the compound identifies a compound having the ability to modulate the activity of Nrf2 by modulating DJ-1 activity.

6. A method of identifying a gene involved in an oxidative stress response, wherein the transcription of said gene is regulated by Nrf2, in a cell, comprising:

a) contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby reducing Nrf2 activity in the cell; and

b) identifying a gene having altered transcription in the cell of step (a), thereby identifying a gene involved in the oxidative stress response of the cell.

7. The method of claim 6, wherein the compound is a small interfering RNA (siRNA).

8. The method of claim 7, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof.

9. A method of modulating an anti-oxidant response in a cell, wherein the anti-oxidant response is directed by DJ-1 or Nrf2, comprising contacting the cell with a compound that alters DJ-1 activity in the cell, thereby altering Nrf2 activity in the cell and modulating the anti-oxidant response in the cell.

10. A method of modulating DJ-1 activity in a cell, comprising contacting the cell with a compound that modulates DJ-1 activity in the cell.

11. A method of modulating Nrf2 activity in a cell, comprising contacting the cell with a compound that modulates DJ-1 activity in the cell.

12. The method of any of claims 9, 10 or 11, wherein the compound is a small interfering RNA (siRNA).

13. The method of claim 12, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof.

14. The method of any of claims 9, 10 or 11, wherein the compound is a nucleic acid molecule encoding a DJ-1 protein.

15. The method of any of claims 9, 10 or 11, wherein the compound is an antibody or ligand that binds DJ-1.

16. The method of any of claims 9, 10 or 11, wherein the compound is a DJ-1 activity-enhancing compound.

17. A method of identifying a subject as having a disorder associated with an altered oxidative stress response, comprising:

a) measuring an amount of DJ-1 activity in a cell of the subject; and

b) comparing the amount of DJ-1 activity in the cell of (a) with the amount of DJ-1 activity in a reference cell, whereby an increased or decreased amount of DJ-1 activity in the cell of (a) as compared to the amount of DJ-1 activity in the reference cell identifies a subject as having a disorder associated with an altered oxidative stress response.

18. A method of identifying a subject with a disorder associated with an altered cellular oxidative stress response as having a poor prognosis, comprising:

a) measuring an amount of DJ-1 activity in a cell of the subject; and

b) comparing the amount of DJ-1 activity in the cell of (a) with the amount of DJ-1 activity in a reference cell, whereby an increased or decreased amount of DJ-1 activity in the cell of (a) as compared to the amount of DJ-1 activity in the reference cell identifies a subject with a disorder associated with an altered oxidative stress response as having a poor prognosis.

19. The method of claim 18, wherein the disorder is selected from the group consisting of lung cancer, colon cancer, prostate cancer, breast cancer and leukemia.

20. A method of identifying a compound useful for treating a disorder associated with an altered oxidative stress response, comprising:

a) contacting the compound with DJ-1 under conditions whereby DJ-1 activity can be measured; and

b) measuring the amount of DJ-1 activity of (a); and

c) comparing the amount of DJ-1 activity of (b) with the amount of DJ-1 activity in the absence of the compound, whereby a decrease or increase in the amount of DJ-1 activity in the presence of the compound as compared to the amount of DJ-1 activity in the absence of the compound identifies a compound useful for treating a disorder associated with an altered oxidative stress response.

21. A method of treating a subject having a disorder associated with an altered oxidative stress response, comprising administering to the subject an effective amount of a compound that modulates DJ-1 activity, thereby modulating Nrf2 activity and treating the disorder associate with an altered oxidative stress response.

22. The method of claim 21, wherein the disorder is cancer, cardiovascular disease, an age-related disorder or a neurodegenerative disorder.

23. The method of claim 21, wherein the compound is selected from the group consisting of oxidative species such as reactive oxygen, nitrogen species, anti-oxidant compounds, tert-butyl hydroquinone, vitamin E, organosulfur compounds, aromatic hydrocarbons, , naturally occurring anti-oxidant compounds and any combination thereof.

24. The method of claim 21, wherein the compound is selected from the group consisting of inhibitors of the anti-oxidative response pathway.

25. The method of claim 21, wherein the compound is a small interfering RNA (siRNA).

26. The method of claim 21, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof.

27. The method of claim 21, wherein the compound is an antibody or ligand that binds DJ-1.

28. The method of claim 21, wherein the compound is an exogenous nucleic acid encoding DJ-1 protein.

29. The method of claim 21, wherein the compound is a DJ-1 activity enhancing compound.

30. A method of identifying a compound that decreases the amount of DJ-1 in a cancer cell in which DJ-1 is produced, comprising:

a) contacting the compound with the cancer cell; and

b) measuring the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell of (a), whereby a decrease in the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the presence of the compound as compared to the amount of DJ-1 mRNA transcript and/or DJ-1 protein in the cancer cell in the absence of the compound identifies a compound that decreases the amount of DJ-1 in the cancer cell in which DJ-1 is produced.

31. A method of identifying a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced, comprising:

a) contacting the compound with the cancer cell; and

b) determining the amount of DJ-1 activity in the cancer cell of (a), whereby an increase or decrease in the amount of DJ-1 activity in the cancer cell in the presence of the compound as compared to the amount of DJ-1 activity in the cancer cell in the absence of the

compound identifies a compound that modulates DJ-1 activity in a cancer cell in which DJ-1 is produced.

32. A method of identifying a cancer cell having increased resistance to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to the amount of DJ-1 and/or Nrf2 activity in a non-cancer cell identifies a cancer cell having an increased resistance to a chemotherapeutic agent.

33. The method of claim 34, wherein the chemotherapeutic agent is selected from the group consisting of Taxol, paclitaxel, MEK kinase inhibitors and any combination thereof.

34. A method of identifying a cancer cell that is susceptible to a chemotherapeutic agent, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell, whereby an increased amount of DJ-1 and/or Nrf2 activity in the cancer cell as compared to a non-cancer cell identifies a cancer cell that is susceptible to a chemotherapeutic agent.

35. The method of claim 34, wherein the chemotherapeutic agent is selected from the group consisting of mitomycin C, Vitamin K3, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1), 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone (MeDZQ) beta-lapachone and any combination thereof.

36. A method of treating a cancer associated with an altered oxidative response in a subject, comprising administering to the subject an effective amount of a compound that modulates DJ-1 activity, thereby modulating Nrf2 activity and treating the cancer associated with an altered oxidative stress response.

37. The method of claim 36, wherein the cancer is selected from the group consisting of lung cancer, prostate cancer, breast cancer, colon cancer and leukemia.

38. The method of claim 36, wherein the compound is a small interfering RNA (siRNA).

39. The method of claim 36, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof.

40. The method of claim 36, wherein the compound is an antibody or ligand that binds DJ-1.

41. The method of claim 36, wherein the compound is a DJ-1 activity-inhibiting compound.

42. A method of identifying a chemotherapeutic agent having a therapeutic effect in a cancer cell, comprising determining the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent, whereby a decrease in the amount of DJ-1 and/or Nrf2 activity in the cancer cell in the presence of the chemotherapeutic agent as compared to the amount of DJ-1 and/or Nrf2 activity in the absence of the chemotherapeutic agent identifies a chemotherapeutic agent having a therapeutic effect in the cancer cell.

43. A method of identifying a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification, comprising:

a) contacting the chemotherapeutic agent with a cell under conditions whereby an Nrf2-mediated detoxification response can occur; and

b) determining if the chemotherapeutic agent has a therapeutic effect on the cell, whereby a chemotherapeutic agent that has a therapeutic effect on the cell in the presence of the Nrf2-mediated detoxification response identifies a chemotherapeutic agent that is resistant to Nrf2-mediated detoxification.

44. A method of treating a cancer in a subject, wherein the cancer is associated with an altered oxidative stress response by inhibiting an anti-oxidant response of an Nrf2-regulated gene, comprising administering to the subject an effective amount of a compound that reduces DJ-1 activity in the cell, thereby reducing Nrf2 activity in the cell, resulting in the downregulation of expression of the Nrf2-regulated gene and an inhibition of the anti-oxidant response of the gene.

45. A method of treating Parkinson's disease in a subject whose cells are deficient in DJ-1 and/or Nrf2 function by inducing expression of Nrf2-regulated genes, comprising administering to the subject an effective amount of an exogenous nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence is expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

46. A method of protecting neurons from destruction in a subject with Parkinson's disease, wherein the cells of the subject are deficient in DJ-1 and/or Nrf2 function, by inducing expression of Nrf2-regulated genes in the subject, comprising administering to the subject an effective amount of an exogenous nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence is expressed to produce the DJ-1 protein, thereby increasing DJ-1 and/or Nrf2 activity in the subject and inducing expression of Nrf2-regulated genes.

47. A method of downregulating the expression of an Nrf2-regulated gene that is associated with an anti-oxidant response in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity, thereby reducing Nrf2 activity, resulting in the downregulation of expression of the Nrf2-regulated gene.

48. The method of claim 47, wherein the Nrf2-regulated gene is selected from the group consisting of NAD(P)H quinone oxidoreductase I (NQO1), heme oxygenase I (HO-I); glucose 6 phosphate dehydrogenase (G6PD), glutathione S-transferase (GST), gluathione cysteine ligase (GCL), superoxide dismutase (SOD), and glutathione S-reductase (GSR) and any combination thereof.

49. The method of claim 47, wherein the compound is a small interfering RNA (siRNA).

50. The method of claim 49, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and a combination thereof.

51. A method of upregulating the expression of an Nrf2-rgulated gene that is associated with an anti-oxidant response in a cell, comprising contacting the cell with a nucleotide sequence encoding a DJ-1 protein under conditions whereby the nucleotide sequence can be expressed to produce DJ-1 protein, whereby the amount of DJ-1 in the cell is increased, resulting in an increase in the amount of Nrf2 in the cell and upregulation of expression of the Nrf2-regulated gene.

52. A method of identifying a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2, comprising:

a) contacting a fragment comprising at least ten contiguous amino acids of the amino acid sequence of the DJ-1 protein with Nrf2 and KEAP1 under conditions whereby binding between Nrf2 and KEAP1 can occur; and

b) determining the amount of Nrf2 bound to KEAP1 in the presence of the fragment as compared to the amount of Nrf2 and KEAP1 bound in the absence of the fragment, whereby a decrease in the amount of Nrf2 bound to KEAP1 in the presence of the fragment as compared to the amount of Nrf2 bound to KEAP1 in the absence of the fragment identifies a domain on the DJ-1 protein that inhibits binding of KEAP1 to Nrf2.

53. A method of identifying a compound that modulates the binding of DJ-1 to a protein selected from the group consisting of amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 4 (ALS2CR4), silent mating type information regulation 2 homolog 7 (sirtuin 7), WD repeat domain 5 (WDR5), plasminogen activator inhibitor 1 RNA binding protein (PAI1RBP; also known as PAI-1 mRNA binding protein and chromodomain helicase DNA binding protein3 interacting protein), eukaryotic translation initiation factor 2, subunit 2 beta, 38 kDa (EIF2S2) and any combination thereof.

54. A method of identifying a compound that modulates the binding of DJ-1 to a protein in the presence of an oxidative stress (e.g., oxidizing H_2O_2), wherein the protein is selected from the group consisting of proapolipoprotein A1, haptoglobin Hp2, lipoprotein CIII, alpha-1-anttrypsin (aa 268-394), amyloid fibril protein-transthyretin-related, glyceraldehydes-3-phosphate dehydrogenase, ADP/ADT translocator protein, human serum albumin in a complex with myristic acid and tri-iodobenzoic acid, ATP-binding cassette, sub family A, member 3, complement component 3 precursor, hypothetical protein MGC20781,

transferrin, heat shock 70 kDa protein 8 isoform 1, hypothetical protein LOC345651, ADP/ATP carrier protein, carbamyl phosphate synthetase I and any combination thereof.

55. A method of measuring the efficacy of a chemotherapeutic agent for treating a cancer in a subject, comprising:

a) measuring the amount of DJ-1 activity in the subject before administering the chemotherapeutic agent to the subject;

b) administering the chemotherapeutic agent to the subject;

c) measuring the amount of DJ-1 activity in the subject during and/or after administering the chemotherapeutic agent to the subject; and

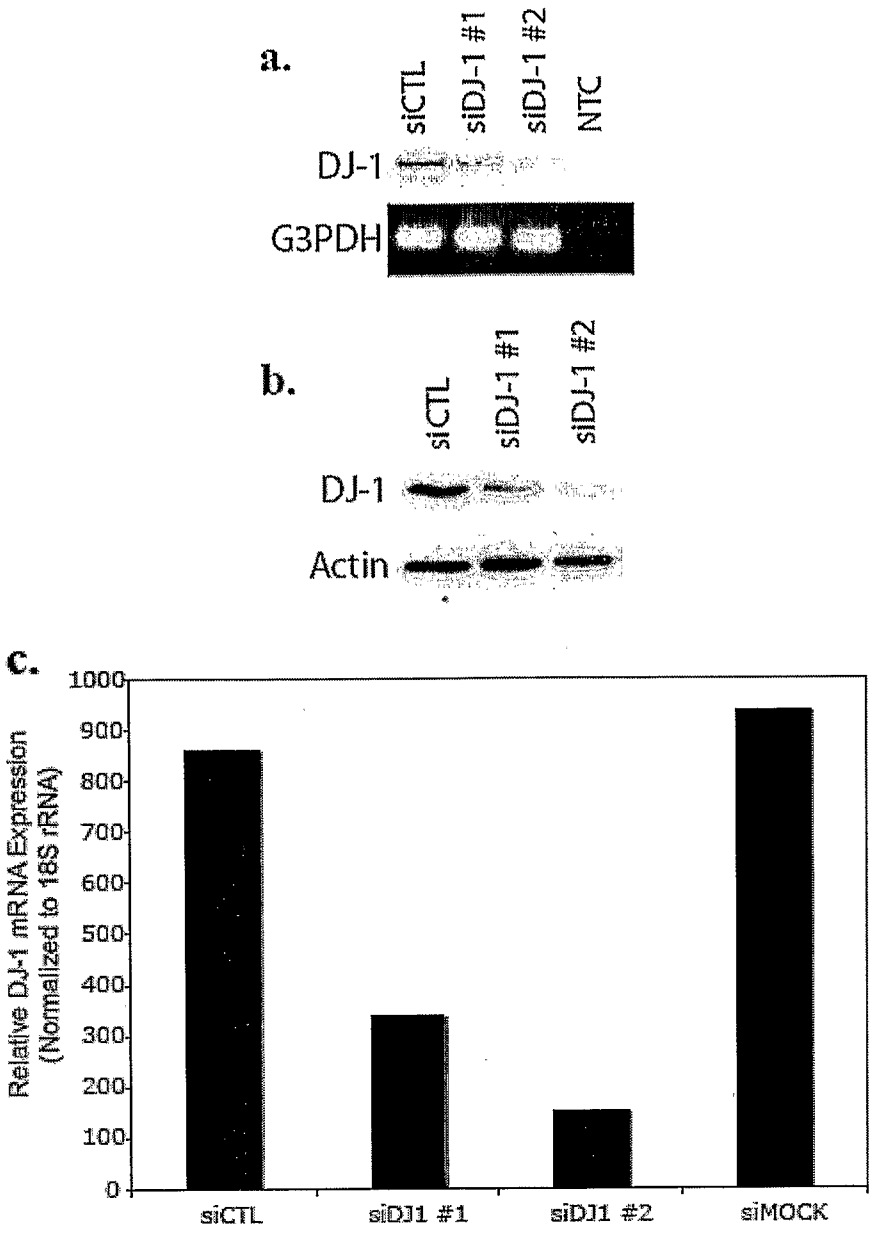
d) comparing the amount of DJ-1 activity of (a) with the amount of DJ-1 activity of (c), whereby a decrease in the amount of DJ-1 activity of (c) identifies a chemotherapeutic agent having efficacy for treating the cancer in the subject.

56. A method of downregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby downregulating expression of the gene.

57. The method of claim 56, wherein the gene is selected from the group consisting of a translocase of outer mitochondrial membrane 20 gene, an RNA polymerase I transcription factor gene, a transmembrance EMP24 transport domain-containing protein gene, a lysosome-associated membrane protein 1 gene, an ATP-binding cassette, subfamily C, member 3 gene, a chloride channel, nucleotide sensitive, 1A gene, an oncogene DJ-1/PARK7 gene, a PC4- and SFRS1-interacting protein 1 gene, a leptin related gene/leptin receptor gene, a Ras-associated protein gene, a phosphatidylinositol glycan, Class B gene, an NAD(P)H dehydrogenase, quinone 1 gene, a HRAS-like suppressor 3 gene, a noggin homolog gene and any combination thereof.

58. A method of upregulating expression of a gene regulated by DJ-1 activity in a cell, comprising contacting the cell with a compound that reduces DJ-1 activity in the cell, thereby upregulating expression of the gene.

59. The method of claim 58, wherein the gene is selected from the group consisting of a gremlin 1 homolog, cysteine knot superfamily gene, a calreticulin gene, a connective tissue growth factor gene and any combination thereof.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NTC
DJ-1
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b.
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DJ-1
Actin
c.
Relative DJ-1 mRNA Expression
(Normalized to 18S rRNA)
1000
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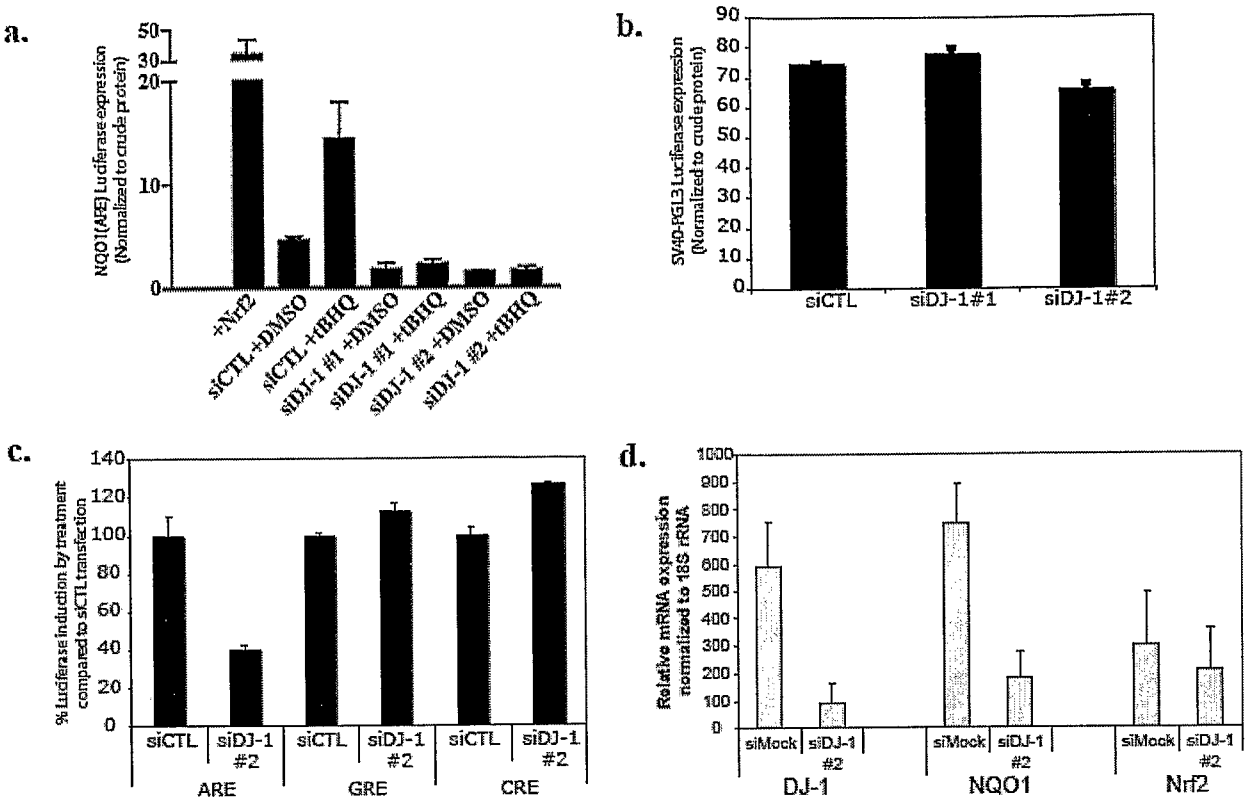
FIGURE 1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30
20
10
0
+Nrf2
siCTL+DMSO
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siDJ-1 #1 +DMSO
siDJ-1 #1 +tBHQ
siDJ-1 #2 +DMSO
siDJ-1 #2 +tBHQ
b.
SV40-PGL3 Luciferase expression (Normalized to crude protein)
90
80
70
60
50
40
30
20
10
0
siCTL
siDJ-1#1
siDJ-1#2
c.
% Luciferase induction by treatment compared to siCTL transfection
140
120
100
80
60
40
20
0
siCTL
siDJ-1 #2
ARE
GRE
CRE
d.
Relative mRNA expression normalized to 18S rRNA
1000
900
800
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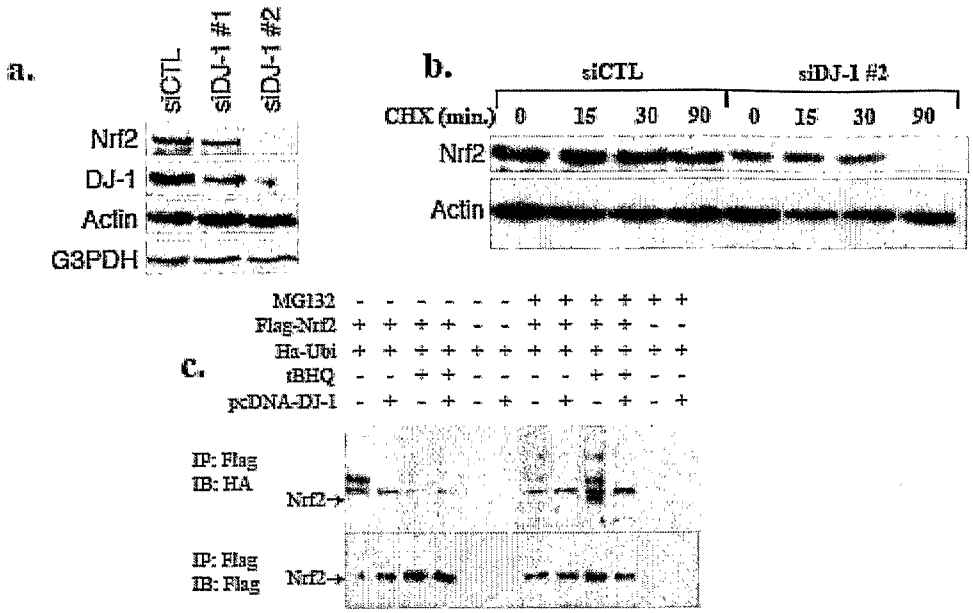
DJ-1
NQO1
Nrf2

FIGURE 2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siDJ-1 #2
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Nrf2
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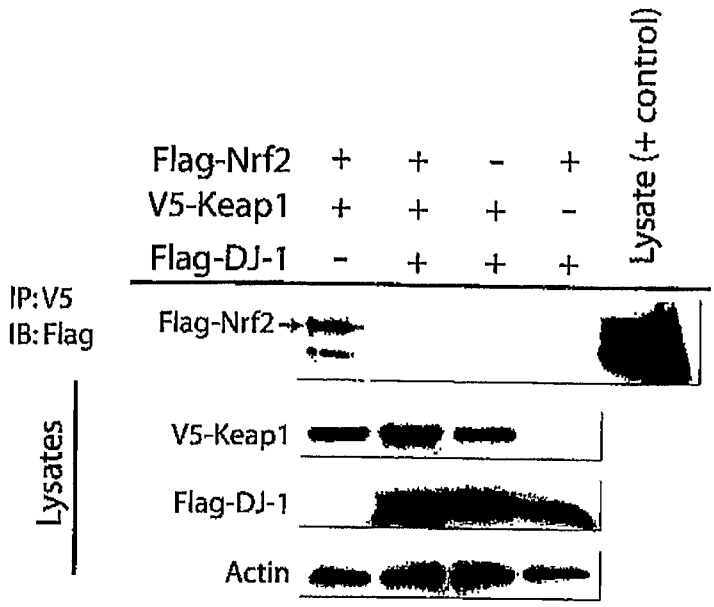
FIGURE 3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V5-Keap1 + + + -
Flag-DJ-1 - + + +
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Lysates
V5-Keap1
Flag-DJ-1
Actin

FIGURE 4

SEQUENCE LISTING

<110> University of North Carolina at Chapel Hill
Ting, Jenny P.-Y.
Clements, Casey

<120> METHODS AND COMPOSITIONS DIRECTED TO DJ-1 AS REGULATOR OF THE ANTI-OXIDANT TRANSCRIPTION FACTOR NRF2

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