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Differential Control of Apoptosis by DJ-1 in Prostate Benign and Cancer Cells

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Abstract DJ-1 is a conserved protein reported to be involved in diverse cellular processes ranging from cellular transformation, control of protein–RNA interaction, oxidative stress response to control of male infertility, among several others. Mutations in the human gene have been shown to be associated with an autosomal recessive, early onset Parkinson's disease (PARK7). The present study examines the control of DJ-1 expression in prostatic benign hyperplasia (BPH-1) and cancer (PC-3) cell lines in which DJ-1 abundance differs significantly. We show that while BPH-1 cells exhibit low basal level of DJ-1 expression, stress-inducing agents such as H_2O_2 and mitomycin C markedly increase the intracellular level of the polypeptide. In contrast, DJ-1 expression is relatively high in PC-3 cells, and incubation with the same cytotoxic drugs does not modulate further the level of the polypeptide. In correlation with the expression of DJ-1, both cytotoxic agents activate the apoptotic pathway in the prostatic benign cells but not in PC-3 cells, which are resistant to their action. We further demonstrate that incubation of BPH-1 cells with TNF-related-apoptosis-inducing-ligand/Apo-2L (TRAIL) also enhances DJ-1 expression and that TRAIL and H_2O_2 act additively to stimulate DJ-1 accumulation but synergistically in the activation of the apoptotic pathway. Time-course analysis of DJ-1 stimulation shows that while DJ-1 level increases without significant lag in TRAIL-treated cells, there is a delay in H_2O_2-treated cells, and that the increase in DJ-1 abundance precedes the activation of apoptosis. Unexpectedly, over-expression of DJ-1 de-sensitizes BPH-1 cells to the action of apoptotic-inducing agents. However, RNA-interference-mediated silencing of DJ-1 expression results in sensitization of PC-3 cells to TRAIL action. These results are consistent with a model in which DJ-1 is involved in the control of cell death in prostate cell lines. DJ-1 appears to play a differential role between cells expressing a low but inducible level of DJ-1 (e.g., BPH-1 cells) and those expressing a high but constitutive level of the polypeptide (e.g., PC-3 cells). J. Cell. Biochem. 92: 1221–1233, 2004.

Key words: DJ-1; apoptosis; TRAIL; prostate

DJ-1 is a 20 kDa protein whose sequence is conserved among prokaryotic and eukaryotic cells [Hod et al., 1999; Wilson et al., 2003]. The exact function of DJ-1 is yet obscure and several diverse cellular roles have been ascribed to it [Cookson, 2003]. DJ-1 was originally reported to be pro-oncogenic, in particular when co-expressed with other oncogenes such as c-myc or c-ras [Nagakubo et al., 1997]. We identified DJ-1 (formally named it RS) as a protein that regulates RNA–protein interaction via its binding to an RNA-binding protein [Nachaliel et al., 1993; Hod et al., 1999], and demonstrated that the interaction of DJ-1 with the RNA-binding protein is regulated by cAMP-mediated phosphorylation [Nachaliel et al., 1993]. Subsequent studies have shown that DJ-1 indirectly regulates the activity of the androgen receptor (AR) in testicular cells via an interaction with PIASxα/ARIP3 (protein inhibitor of activated STATxα/androgen receptor-interacting protein 3) [Takahashi et al., 2001], an E3-like enzyme that adds SUMO-1 to target proteins, as well as with DJBP—a novel DJ-1 binding protein [Niki et al., 2003]. Both PIASxα/ARIP3 and DJBP independently block AR transactivation, but their interaction with DJ-1 abrogates the inhibition of the AR. In addition to the role of DJ-1

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in testicular cells, the polypeptide (designated in some studies as either SP22 or CAP1) was found in the sperm of several mammalian species [Welch et al., 1998; Wagenfeld et al., 2000; Whyard et al., 2000; Klinefelter et al., 2002; Yoshida et al., 2003]. Incubation of isolated sperm with specific antibodies to DJ-1 resulted in diminished capacity to fertilize both in vivo and in vitro [Klinefelter et al., 2002; Okada et al., 2002], suggesting that DJ-1 also plays an important role in fertilization.

Employing proteomic profiling, DJ-1 was found in the sera of a large fraction (37%) of newly diagnosed patients with breast cancer, as well as a secreted protein in the growth medium of a malignant breast cancer cell line [Le Naour et al., 2001]. Other proteomic studies have found the level of DJ-1 to decline in a non-small lung carcinoma induced to undergo apoptosis [MacKeigan et al., 2003], and to differentially increase during the re-epithelialization process of wound healing [El Ghalbzouri et al., 2004]. In addition, DJ-1 levels were enhanced in primary lung tumors [MacKeigan et al., 2003] and in prostate cancer [Grzmil et al., 2004], indicating that DJ-1 expression increases with cell growth and transformation.

The human *DJ-1* gene is located on the distal part of the short arm of chromosome 1 (1p36.12-1p36.33). Mutations in the gene have been reported to be linked with a form of autosomal receive early-onset familial Parkinson's disease (PD) [Bonifati et al., 2003]. This motoric disorder is characterized by the loss of dopamine neurons in the substantia nigra, the region in the brain that controls movement [Olanow and Tatton, 1999]. Two types of mutations in the *DJ-1* gene were reported in two independent families [Bonifati et al., 2003]: in one, there is loss of a ~14 kb of the 5′-region of the gene including the promoter region and the most 5′-five exons, while in the other family there is a point mutation resulting in a L166P substitution. Based on crystallization analysis of the purified human protein [Honbou et al., 2003; Huai et al., 2003; Lee et al., 2003; Tao and Tong, 2003; Wilson et al., 2003], DJ-1 was proposed to exist as a dimer and that the L166P mutation destabilizes the dimer interface. A more direct analysis of a recombinant L166P protein expressed in mammalian transfected cells showed that the indicated mutation promotes DJ-1 degradation through the ubiquitin-proteosome system, resulting in a low level of the protein [Macedo et al., 2003; Miller et al., 2003; Olzmann et al., 2004].

The neuronal function of DJ-1 and the pathogenesis of PD due to DJ-1 loss of activity are still unknown. A prevailing hypothesis is that oxidative stress and dysfunction of the ubiquitin-proteosome system may be the cause for the selective degeneration of dopaminergic neurons in PD [Cookson, 2003; Dawson and Dawson, 2003; Jenner, 2003]. The potential involvement of DJ-1 in these pathways is suggested by the fact that mutations in three other genes that promote PD (α-synuclein, parkin, and UCLH1) [Cookson, 2003; Dawson and Dawson, 2003; Hattori et al., 2003], resulted in protein aggregation due to oxidative stress and proteosomal dysfunction. Dawson and Dawson [2003] have suggested that DJ-1 may function as a chaperon, while Olzmann et al. [2004] demonstrated that DJ-1 is a protease. Whether DJ-1 has a chaperon or protease activity is still controversial [Lee et al., 2003; Wilson et al., 2003; Olzmann et al., 2004]. In addition, a recent study has shown that DJ-1 protects neuroblastoma cells in culture against oxidative stress and that it has the capacity, at least in vitro, to act and eliminate H_2O_2 [Taira et al., 2004].

DJ-1 is ubiquitously expressed in many, if not all, mammalian tissues [Nagakubo et al., 1997]. In the present study, we tested the control of DJ-1 expression in prostate cells because early screening of the level of DJ-1 in different cell lines showed that its abundance is significantly lower in a cell line derived from a benign prostate hyperplasia (BPH-1) relative to that in several prostate cancer cell lines. We focused first on drugs that induce oxidative damage because exposure of human epithelial cells to sub-lethal levels of compounds that produce reactive oxygen species (ROS) such as H_2O_2 and paraquat, resulted in a shift in the protein to a more acidic isoform [Mitsumoto et al., 2001], suggesting that DJ-1 is an oxidative sensor. Similar results were noted in endotoxin-mediated inflammation-induced macrophages, a condition known to endogenously generate ROS [Mitsumoto and Nakagawa, 2001]. We demonstrate here that the level of DJ-1 is stimulated in BPH-1 cells treated with H_2O_2 as well as with other non-oxidative stress-inducing drugs. The level of DJ-1 was also increased in cells incubated with TNF-related apoptosis-inducing ligand/Apo-2L (TRAIL). The

pattern of DJ-1 expression in response to these stimuli is consistent with a mechanism in which stimulation of DJ-1 levels is associated with the activation of cell death. In contrast, the prostate cancer PC-3 cells which express a high, but constitutive level of DJ-1, are resistant to the same treatments of stress- and apoptosis-inducing drugs. Over-expression of DJ-1 de-sensitizes BPH-1 cells to apoptosis-inducing agents while siRNA-mediated arrest of DJ-1 sensitizes PC-3 cells to the same treatments. We suggest that DJ-1 is a stress protein and discuss its potential role in the control of apoptosis.

MATERIALS AND METHODS

Cell Cultures

The prostate cancer cell lines LNCaP, Du-145, and PC-3 were purchased from American Type Culture Collection (ATCC, Manassa, VA). The non-tumorigenic human benign prostatic hyperplasia BPH-1 cell line was obtained from Dr. Simon W. Hayward (Vanderbilt University, Nashville, Tennessee). The establishment and characterization of the BPH-1 cell line was described [Hayward et al., 1995]. All cell lines were maintained in RPMI 1640 medium (Cellgro, Herndon, VA) supplemented with 10% Cosmic Calf Serum (HyClone, Logan, UT) in a 5% CO_2-humidified tissue culture incubator. All experiments were performed when cultures reached 70–80% confluency. Cell extracts were prepared by scraping the cells in the incubation medium followed by two washes in cold phosphate-buffered saline and lysis in medium composed of Tris-HCl (50 mM, pH 7.4), NaCl (150 mM), NP-40 (1%), Na-deoxycholate (0.25%), EDTA (1 mM), Na_3VO_4 (1 mM), NaF (1 mM) containing a mixture of protease inhibitors (Sigma-Aldrich, Inc., St. Louis, MO, Catalog no. P-8340). Following 10 min at 4°C and centrifugation at 13,000*g* for 15 min, protein concentration in the clear supernatant was measured employing the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

Production of Monoclonal Antibodies to DJ-1

Monoclonal antibodies to DJ-1 were prepared essentially as described [Harlow and Lane, 1988] except that 250 μg of the purified full length recombinant DJ-1 [Hod et al., 1999] in complete Freund's adjuvant was subcutaneously injected per mouse, followed by one additional booster injection of 50 μg, 21 days later. The reactivity of the finally isolated monoclonal antibody (mαDJ-1/E2.19) was verified by demonstrating that it specifically recognized a fusion protein of GST/DJ-1, but not GST alone (not shown), as well as a FLAG-tagged DJ-1 expressed in eukaryotic cells (for example, see Fig. 7). The antibody appears to be of the IgM class.

SDS–PAGE and Western Blot Analysis

SDS–PAGE was carried out under standard conditions and proteins were electroblotted onto a PVDF membrane (PolyScreen, PerkinElmer Life Sciences, Inc., Boston, MA) employing a GENIE Blotter (Idea Scientific, Minneapolis, MN). Blots were blocked with nonfat milk and incubated for 1 h at room temperature with either mαDJ-1/E2.19 (1:500 dilution), or with a monoclonal antibody to cleaved PARP (Asp124) (clone 19F4) (Cell Signaling Technology, Inc., Beverly, MA) (1:2,000 dilution), each together with a monoclonal antibody to β-actin (clone AC-74) (Sigma-Aldrich, Inc.) (1:5,000 dilution). After washing and 1 h incubation with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (whole molecule) (1:5,000 dilution) (Sigma-Aldrich, Inc.), blots were developed using the SuperSignal enhanced chemiluminescence detection kit from Pierce at 1:10 dilution. All experiments were carried out under conditions whereby the intensity of the detected signals was co-linear with the amount of input protein loaded on the gel.

Construction and Expression of a FLAG-DJ-1 Fusion Protein

DJ-1 cDNA (AF021819) was amplified by PCR under standard conditions using the upstream DNA primer 5′-CATAAAAATGAATTCGAAAAGAGCTCTGG and the downstream primer 5′-CTGTTTCTCGAGGTGATCGTCGCAGTTCGCTG. The amplified DNA was digested with Eco*RI* and Xho*I* (sequence underlined) and inserted into the corresponding sites of the eukaryotic expression vector pCMV-Tag2 (Stratagene, La Jolla, CA). The resulting DNA (designated DJ-1/F) placed the sequence encoding the FLAG epitope upstream of the complete coding sequence of the human DJ-1 starting with the codon for the third amino acid. The purified DNA (3.25 μg) was mixed with a construct carrying a gene that confers hygromycin resistance (pTK-Hyg) (BD Bioscience Clontech, Palo Alto, CA) (0.75 μg) and introduced into

BPH-1 cells using Lipofectamine™ 2000 reagent (Invitrogen Life Technologies, Carlsbad, CA) as instructed by the supplier. Control cells were transfected with the pCMV-Sport β-gal, (Invitrogen Life Technologies), a vector coding for β-galactosidase, together with the pTK-Hyg construct. Transfected cells were selected in medium containing hygromycin (160 μg/ml) and resistant colonies were expanded. Western blotting analyses employing the antibodies to DJ-1 or β-galactosidase were used to identify positively expressing cells.

Construction and Expression of Small Interference RNA

The mammalian expression vector, pSUPER (OligoEngine, Seattle, WA) was used for expression of siRNA in PC-3 cells. The human *DJ-1* gene-specific double-stranded fragment that specifies a 19-nucleotide sequence corresponding to nucleotides 193–211 downstream of the transcription start site (5′-GACCCAGTACAGTGTAGCC) and separated by a 9-nucleotide non-complementary spacer from the reverse complement of the same 19-nucleotide sequence was synthesized and inserted between the Bgl II and Hind III sites of the vector. The selected sequence did not match any sequences other than DJ-1 in GenBank™. The original pSUPER vector served as a non-silencing control. Each of the purified constructs (4 μg) was combined with a vector coding for the neomycin gene (pSV40-neo) (1 μg) and introduced into PC-3 cells employing Lipofectamine 2000 (Invitrogen Life Technologies) as instructed. Cells were exposed to G418 (400 μg/ml) 48 h following transfection, and isolated colonies were randomly selected and expanded. Western blotting analysis was employed to determine DJ-1 expression in selected colonies.

RESULTS

Differential Expression of DJ-1 in Prostate Benign and Cancer Cell Lines

Figure 1 shows Western blot analysis of protein extracts from a BPH-1 cell line and from several prostate cancer cell lines including the androgen-responsive LNCaP and the metastatic androgen-resistant Du145 and PC-3 cell lines. Densitometric scanning of the autoradiogram (not shown) demonstrated 8–13 fold increase in the level of DJ-1 in the prostate cancer cells relative to that in BPH-1 cells. In the present study, we have selected PC-3 cells to represent the group of cell lines expressing a high level of DJ-1.

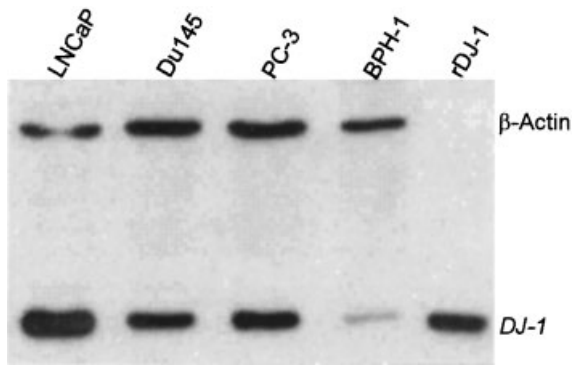

Fig. 1. Expression of DJ-1 in different prostate cell lines. Western blot analysis of DJ-1 using protein extracts (6 μg/lane) from the cell lines LNCaP; Du145; PC-3; and BPH-1, as indicated. rDJ-1 is a recombinant form of the full-length DJ-1 expressed in *E. coli* and purified as described [Hod et al., 1999]. Forty nanograms of rDJ-1 was loaded onto the indicated lane. The blot was co-reacted with the monoclonal antibodies to DJ-1 and β-actin as described under ''Materials and Methods.'' The position of DJ-1 and β-actin is indicated.

Figure 2 (top panels) shows a dose response of BPH-1 and PC-3 cells to increasing concentrations of H_2O_2. While exposure of BPH-1 cells to H_2O_2 at 50 μM was sufficient to significantly enhance DJ-1 level, even a concentration as high as 500 μM did not modulate DJ-1 level in PC-3 cells. To test whether the effect is limited to H_2O_2, we examined the expression of DJ-1 in cells incubated with mitomycin C (MMC) (Fig. 2, bottom panels). MMC is a pro-drug that is activated in vivo by enzymatic reduction yielding products capable of cross-linking DNA strands [Tomasz et al., 1987]. As with H_2O_2, while incubation of BPH-1 cells with MMC at concentrations as low as 0.5–10 μg/ml stimulated DJ-1 level, no such modulation was noted in PC-3 cells in response to MMC treatment. These results demonstrate that while the basal level of DJ-1 in BPH-1 cells is relatively low, its expression is inducible, in contrast to PC-3 cells that exhibit a high level and constitutive expression of the protein.

H_2O_2 and MMC Induce Apoptosis in BPH-1 but not in PC-3 Cells

Cells exposed to severe oxidative or any other toxic stress are likely to die. We therefore examined whether H_2O_2 and MMC also induced proapoptotic signals. We used the cleavage of the 116 kDa poly(ADP-ribose) polymerase (PARP) to an 89 kDa fragment as a measure of

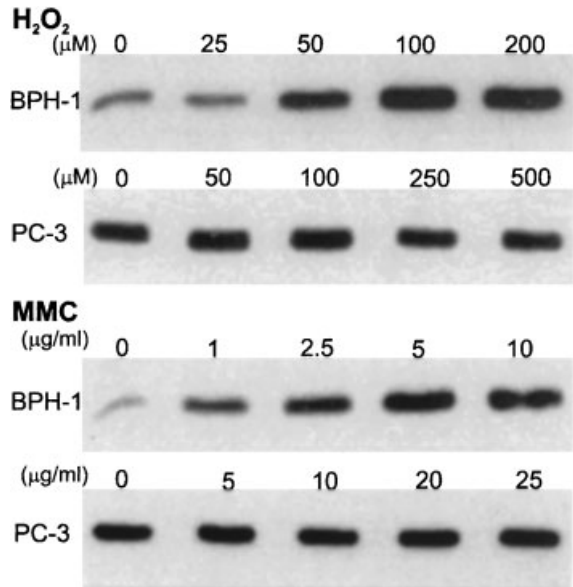

Fig. 2. H_2O_2- and MMC-control of DJ-1 expression in BPH-1 and PC-3 cells. Western blot analyses of DJ-1 level in BPH-1 and PC-3 cells treated with H_2O_2 (**top panels**) or MMC (**bottom panels**) at the indicated concentrations. Cells were incubated with H_2O_2 or MMC for 18 h. Protein extracts (20 μg/lane for BPH-1 cells and 6 μg/lane for PC-3 cells) were separated by electrophoresis on a 12% polyacrylamide gel in the presence of SDS (0.2%). The blots were also reacted with an antibody to β-actin. No significance differences in β-actin levels were noted among the different samples in each of the blots (not shown). All other details are described under ''Materials and Methods.''

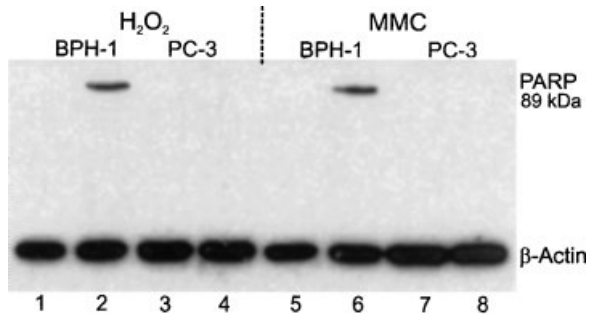

Fig. 3. Proteolytic cleavage of PARP in PBH-1 and PC-3 cells incubated with H_2O_2 and MMC. Western blot analysis of PARP cleavage in BPH-1 (**lanes 1**, **2**, **5**, **6**) and PC-3 (**lanes 3**, **4**, **7**, **8**) cells. Cells were exposed to H_2O_2 at 200 μM (lane 2) or 500 μM (lane 4) for 18 h and to MMC at 10 μg/ml (lane 6) or 25 μg/ml (lane 8) for 18 h. Cells in lanes 1, 3, 5, and 7 were incubated in control medium. The blot was reacted with the monoclonal antibodies to PARP and β-actin. The position of the cleaved form of PARP (89 kDa) and that of β-actin is indicated. Protein extracts (20 μg/lane for both BPH-1 and PC-3 cells) were separated by electrophoresis on an 8% polyacrylamide gel in the presence of SDS (0.2%). All other details are described under ''Materials and Methods.''

cells undergoing cell death. The site-specific cleavage of PARP is catalyzed by caspases [Nicholson et al., 1995], a step considered one of the early biochemical events in apoptotic cell death [Oliver et al., 1998]. As seen in Figure 3, while both H_2O_2 and MMC induced PARP cleavage in BPH-1 cells (lanes 1, 2 and 5, 6), PC-3 cells were resistant to the actions of these cytotoxic drugs (lanes 3, 4 and 7, 8). An analysis of cell death by DNA fragmentation (not shown) demonstrated a similar pattern of results. Later experiments (see Fig. 8) showed that PARP cleavage could be stimulated in PC-3 cells, ruling-out the possibility that PARP is not accessible to caspase action in these cells. Considering the results of Figures 2 and 3, it appears that the differential sensitivity of BPH-1 and PC-3 cells to the cytotoxic actions of H_2O_2 and MMC correlates with the differential level of expression of DJ-1, and its inducibility, in these prostate cell lines.

TRAIL Stimulates DJ-1 Level in BPH-1 Cells

To elucidate the role of DJ-1 in the response to cellular stress, we used TRAIL to test whether a highly specific apoptosis-inducing agent also modulates the level of DJ-1. Activation of apoptosis by TRAIL is mediated by binding of the ligand to death domain-containing receptors (DR4 and DR5) leading eventually to the activation of effector caspases such as caspase-3 and -7 [Suliman et al., 2001]. Figure 4A shows dose-response of BPH-1 cells treated with increasing concentrations of TRAIL. Densitometric scanning of the autoradiogram (not shown) showed about 6-fold increase in DJ-1 level in cells treated with 250 ng/ml of TRAIL relative to that in control cells, demonstrating that DJ-1 expression is also regulated by a specific agent that induces apoptosis. To determine whether the changes in DJ-1 level in cells treated with TRAIL and H_2O_2 are mediated by related mechanisms, we tested the expression of DJ-1 in BPH-1 cells co-incubated with a limited concentration of H_2O_2 (20 μM) in the presence of increasing concentrations of TRAIL. As seen in Figure 4B, the magnitude of the increase in the level of DJ-1 in cells treated with both H_2O_2 and TRAIL was the additive effect of each of these agents, suggesting that they stimulated DJ-1 expression by the same mechanism. In contrast, H_2O_2 and TRAIL acted synergistically on PARP cleavage (Fig. 4C), suggesting that the activation of the apoptotic pathway in BPH-1 cells by H_2O_2 and TRAIL is mediated by independent mechanisms.

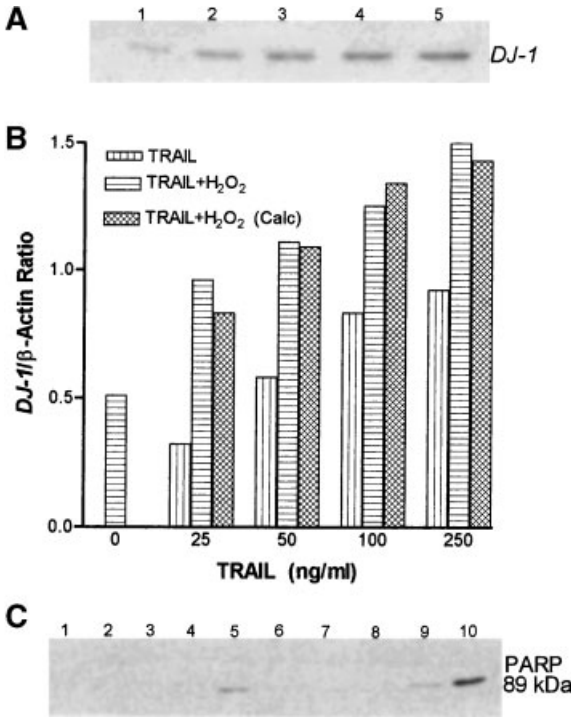

Fig. 4. TRAIL-mediated DJ-1 accumulation in BPH-1 cells. **A**: Western blot analysis of DJ-1 expression in BPH-1 cells incubated in medium containing TRAIL at 0, 25, 50, 100, and 250 ng/ml (**lanes 1–5**, respectively) for 18 h. **B**: DJ-1 levels, as depicted by Western blot analysis, in BPH-1 cells incubated for 18 h in medium containing either TRAIL alone at the indicated concentration or in the presence of the same TRAIL concentrations together with H_2O_2 (20 μM). The value for the DJ-1/β-actin ratio (0.19) for cells incubated in control medium (without TRAIL or H_2O_2) was subtracted from experimental data. [TRAIL + H_2O_2 (Calc)] is an estimated value for DJ-1/β-actin ratio for cells treated with TRAIL together with H_2O_2 calculated by adding the value for cells incubated with H_2O_2 alone ('0' TRAIL) with each of the values measured for the indicated TRAIL concentration. **C**: Western blot analysis of PARP cleavage in BPH-1 cells incubated for 18 h in medium containing either TRAIL alone (lanes 1–5) or TRAIL together with H_2O_2 (20 μM) (**lanes 6–10**). TRAIL concentrations were: '0' (lanes 1 and 6); 25 (lanes 2 and 7); 50 (lanes 3 and 8); 100 (lanes 4 and 9); and 250 ng/ml (lanes 5 and 10). The blots in both A and C were also reacted with an antibody to β-actin. No significance differences in β-actin levels were noted among the different samples in each of the blots (not shown). For SDS–PAGE analysis of DJ-1 and PARP, protein extracts (20 μg/lane) were separated by electrophoresis on 12 and 8% polyacrylamide gels, respectively. All other details are described under ''Materials and Methods.''

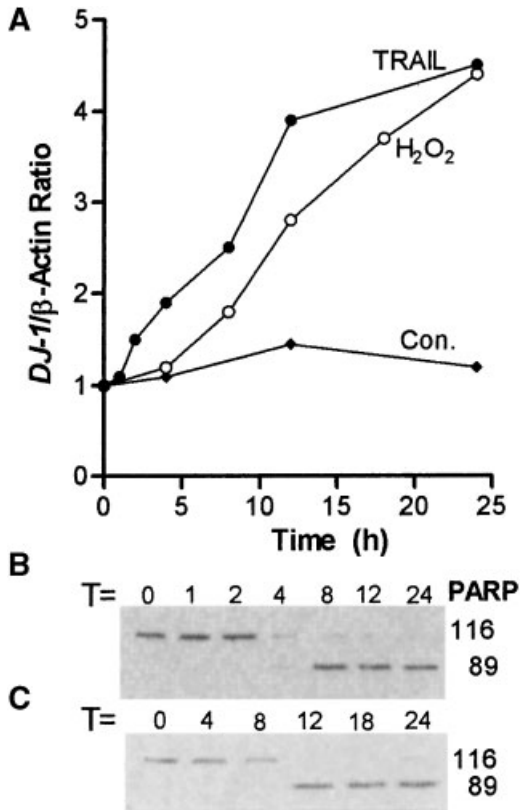

Fig. 5. Time-course of DJ-1 accumulation in BPH-1 cells treated with TRAIL or H_2O_2. **A**: DJ-1 levels in BPH-1 cells incubated for the indicated times with either TRAIL (250 ng/ml) (closed circles), or H_2O_2 (200 μM) (open circles), or under control conditions (closed diamond), as depicted by Western blotting. **B** and **C** show time-course analyses of PARP cleavage in cells exposed to TRAIL (250 ng/ml) (B) or H_2O_2 (200 μM) (C) for the indicated times (h). The mature size PARP (116 kDa) and its cleaved form (89 kDa) are indicated. For SDS–PAGE analysis of DJ-1 and PARP, protein extracts (20 μg/lane) were separated by electrophoresis on 12 and 8% polyacrylamide gels, respectively. Blots were reacted also with monoclonal antibodies to β-actin and no significance differences were noted among the different samples in each of the blots (not shown). All other details are described under ''Materials and Methods.''

Time-Course Analysis of DJ-1 Stimulation by TRAIL and H_2O_2

Figure 5A shows a time-course of DJ-1 accumulation in BPH-1 cells treated with either TRAIL or H_2O_2. While the rise in the level of DJ-1 started without significant time lag in TRAIL-treated cells, there was a consistent 4–8 h lag before DJ-1 started to accumulate in cell exposed to H_2O_2. The noted delay in H_2O_2-treated cells suggests that DJ-1 is unlikely to be a scavenger enzyme because such enzymes are expected to be activated as soon as cells are exposed to damaging insults. As also seen in Figure 5B and C, PARP processing under the above conditions followed the changes in DJ-1 level. While the 89 kDa form of PARP first appeared at a detectable level at the 4 h time-point in TRAIL-treated cells (Fig. 5B), more than 8 h elapsed before it was first detected in

the H_2O_2-treated cells (Fig. 5C), showing that the increase in the level of DJ-1 precedes the cleavage of PARP. A similar delay was seen in BPH-1 cells exposed to MMC (not shown), suggesting that the effect is not drug-specific.

To re-assess the delay in DJ-1 stimulation in cells exposed to H_2O_2, we examined the exposure time required to induce maximum level of the polypeptide. In this experiment, BPH-1 cells were incubated in medium containing H_2O_2 for 1–24 h, and the level of DJ-1 was measured at the 24 h time point, after the H_2O_2-containing medium was washed off. As seen in Figure 6A, H_2O_2 exposure for 1 h was sufficient to induce a maximum level of DJ-1 at 24 h, suggesting that prolonged presence of H_2O_2 is not necessary to stimulate DJ-1 level and that the inducing effect persists even after the removal of the cytotoxic agent. PARP cleavage followed the changes in DJ-1 and 1 h was sufficient to activate PARP cleavage (Fig. 6B). Taken together, the delay in DJ-1 stimulation is consistent with a mechanism in which DJ-1 does not play a direct role in the actual protective mechanism against cellular damage but rather a delayed role as a consequence of a cytotoxic injury.

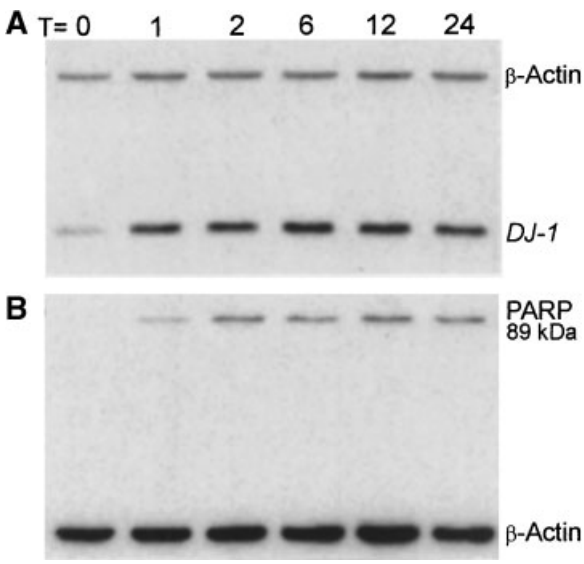

Fig. 6. Exposure time to H_2O_2 required to stimulate DJ-1 level in BPH-1 cells. Western blot analysis of DJ-1 (**A**) and PARP (**B**) in cells incubated with H_2O_2 (200 μM) for the indicated times (h). Following the incubation with H_2O_2 and two washes, cells were exposed to growth medium and incubation was continued for the remaining time up to 24 h. For SDS–PAGE analysis of DJ-1 and PARP, protein extracts (20 μg/lane) were separated by electrophoresis on 12 and 8% polyacrylamide gels, respectively. All other details are described under ''Materials and Methods.''

Over-Expression of DJ-1 De-Sensitizes BPH-1 Cells to the Induction of Apoptosis by H_2O_2

To further link the expression of DJ-1 with the control of cell death, we have constructed and isolated a sub-line of BPH-1 cells expressing FLAG-tagged DJ-1 (designated DJ-1/F), the transcription of its gene was driven by a CMV promoter (see, Material Methods). Western blot analysis showed about 3-fold increase in the total basal level of DJ-1 in the DJ-1/F-expressing cells relative to control cells expressing the endogenous gene (Fig. 7A). Exposure of cells to increasing concentrations of H_2O_2 resulted in parallel increase in the level of both DJ-1 and DJ-1/F, suggesting that the enhancement in their levels could have been mediated by a mechanism other than a stimulation of gene transcription. Unexpectedly, the cleavage of PARP was partially blocked in H_2O_2-treated cells over-expressing DJ-1/F relative to cells expressing the endogenous gene (Fig. 7B). Similar resistance to the activation of PARP cleavage was noted in cells incubated with TRAIL (200 ng/ml) (not shown) suggesting that a high level of DJ-1 may confer cells with resistance to apoptosis-inducing drugs. Although these results may appear in conflict with others obtained in experiments employing BPH-1 cells, we noted that the loss of drug-sensitivity is reminiscent of PC-3 cells shown earlier to exhibit a high level of DJ-1 and reduced sensitivity to cytotoxic drugs (Figs. 2 and 3).

siRNA-Mediated Down-Regulation of DJ-1 Sensitizes PC-3 Cells to TRAIL

We employed siRNA to test the consequence of reduced DJ-1 expression on TRAIL-mediated induction of apoptosis in PC-3 cells. DNA encoding a DJ-1 sequence-specific siRNA in a stem-loop structure under the control of the H1-RNA promoter was constructed as described under "Materials and Methods." This construct (designated P/Si), as well as an 'empty' vector (designated P/Sp), were introduced into PC-3 cells and several independent colonies were selected and expanded, as described under "Materials and Methods." Among the cells transfected with the DJ-1 siRNA sequence, five out of six were found to have a significantly reduced level of DJ-1 relative to that in control cells (Fig. 8A).

Figure 8B shows Western blot analysis of PARP in P/Sp1 and P/Si3 cells incubated for

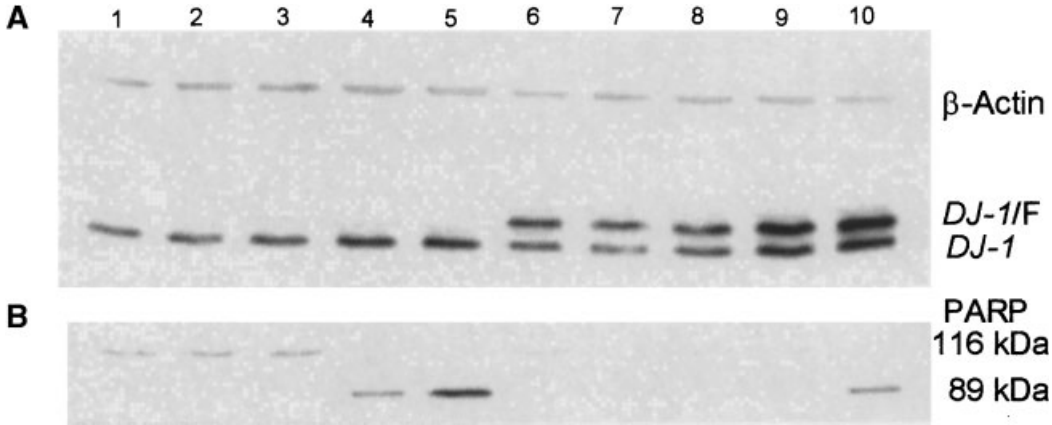

Fig. 7. Over-expression of DJ-1 de-sensitizes BPH-1 cells to H_2O_2. Western blot analysis of DJ-1 (**A**) and PARP (**B**) in control cells (**lanes 1–5**) or cells expressing DJ-1/F (FLAG-tagged DJ-1) (**lanes 6–10**). Cells were incubated for 18 h either under control conditions in medium without H_2O_2 (lanes 1 and 6) or in medium containing 25 (lanes 2 and 7), 50 (lanes 3 and 8), 100 (lanes 4 and 9) and 200 μM H_2O_2 (lanes 5 and 10). For SDS–PAGE analysis of DJ-1 and PARP, protein extracts (20 μg/lane) were separated by electrophoresis on 12 and 8% polyacrylamide gels, respectively. All other details are described under ''Materials and Methods.''

18 h with increasing concentrations of TRAIL (50–500 ng/ml). While TRAIL induced apoptosis in P/Si3 cells incubated with a concentration as low as 50 ng/ml, no PARP cleavage was noted in P/Sp1 cells even at a concentration as high as 500 ng/ml. An experiment with other sub-lines of P/Si (P/Si1 and P/Si4) showed similar cleavage of PARP in cultures treated with TRAIL (100 ng/ml) in contrast to a second control subline (P/Sp2) that was resistant (not shown). We also found that the effect of TRAIL was relatively fast and the cleaved form of PARP was detected in P/Si3 cells as early as 4 h after their exposure to the ligand (not shown), a time-course reminiscent of that seen in BPH-1 cells (Fig. 5). We concluded that while DJ-1 is involved in the control of cell death in prostate cells, its role is complex: it confers resistance to apoptotic-inducing agents to cells expressing a high level of the polypeptide whereas stimulation in DJ-1 level appears to be associated with the induction of apoptosis in cells expressing low level of DJ-1.

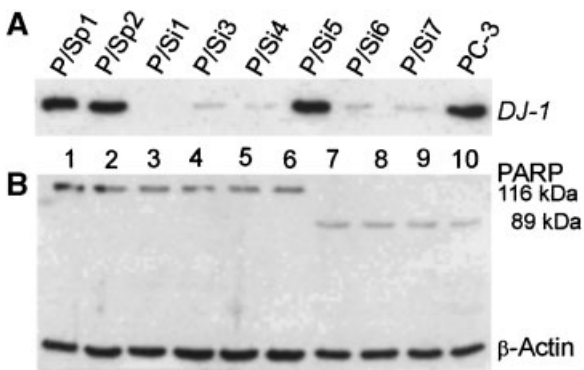

Fig. 8. siRNA-mediated DJ-1 silencing sensitizes PC-3 cells to TRAIL. **A**: Western blot analysis of DJ-1 in independent colonies of PC-3 cells transfected with either P/Si (a vector encoding DJ-1-specific siRNA) or P/Sp (an 'empty' vector). The colonies with the designated numerical are indicated. The 'PC-3' lane contains an extract from non-transfected PC-3 cells. **B**: Western blot analysis of PARP cleavage in P/Sp2 (**lanes 1–5**) and P/Si3 (**lanes 6–10**) incubated for 18 h either under control conditions in medium without TRAIL (lanes 1 and 6) or in medium containing TRAIL at 50 (lanes 2 and 7), 100 (lanes 3 and 8), 250 (lanes 4 and 9), and 500 ng/ml (lanes 5 and 10). All other details are described under ''Materials and Methods'' except that protein extracts were loaded at 6 and 20 μg/lane on a 12 and 8% polyacrylamide gels for DJ-1 and PARP analyses, respectively.

DISCUSSION

The present study shows that DJ-1 plays a direct role in the control of apoptosis in human prostate cells. This conclusion is supported by the demonstration that DJ-1 expression was modulated in a dose-response manner in BPH-1 cells exposed to a specific apoptosis-inducing agent (TRAIL) (Fig. 5A) and that siRNA-mediated reduced expression of DJ-1 sensitized the otherwise resistant PC-3 cells to the same apoptotic-inducing ligand (Fig. 8). TRAIL is a transmembrane protein that shares homology in its extracellular domains with other members of the TNF family of apoptosis-inducing ligand. The ligand has been shown to trigger apoptosis in a wide variety of transformed and cancerous cells via interaction with specific

death domains-containing receptors (DR4 and DR5) [see Ashkenazi and Dixit, 1998, 1999 and references therein]. Upon activation, the death receptors initiate a cascade pathway that leads to the proteolytic activation of the effector caspase-3 [Ashkenazi and Dixit, 1999]. Caspase-3 is responsible in turn, either alone or together with other effector caspases, for the cleavage of several cytoskeletal and nuclear proteins like PARP, α-fodrin, and lamin A [Wolf and Green, 1999], leading eventually to cell disassembly and death. The potential role of DJ-1 in the control of cell death was suggested earlier by the demonstration that induction of apoptosis in a non-small cell lung carcinoma resulted in concomitant marked decline in the level of two proteins, one of which was DJ-1 [MacKeigan et al., 2003]. Taken together, these results strongly suggest that DJ-1 is involved in the control of cell death in mammalian cells.

The present study also demonstrates that stress-inducing agents such as H_2O_2 and MMC stimulate the level of DJ-1 in the BPH-1 cells and that the changes in DJ-1 level correlate with the induction of apoptosis in these cells. While H_2O_2 was reported to have the potential to directly attack specific enzymes [Brodie and Reed, 1987], its major cytotoxic effect is known to be mediated by hydroxyl radicals ($OH^{\bullet}$) generated by the reaction of H_2O_2 with intracellular stores of iron [Goldstein et al., 1993]. Addition of H_2O_2 to mammalian cells produces increased strand breakage within a few minutes [Spencer et al., 1995] either by the direct attack of the highly reactive hydroxyl radicals on DNA [Spencer et al., 1995] or indirectly by a rise in intracellular free Ca^{2+} that is sufficient to activate endonucleases [Orrenius et al., 1989]. MMC is a known anticancer drug that is activated by enzymatic reduction [Sartorelli et al., 1993] producing a highly reactive intermediate that binds and cross-links DNA [Tomasz et al., 1987]. The stimulation in the level of DJ-1 in BPH-1 cells treated with either H_2O_2 or MMC shows that DJ-1 is more than an oxidative stress sensor (or hydroperoxide-responsive protein) as suggested [Mitsumoto and Nakagawa, 2001; Mitsumoto et al., 2001; Taira et al., 2004], but rather a protein that plays a broader role in response to cellular damage.

The cellular response to stress-inducing agents may be sub-divided into three major steps. The first step entails a protective response that aims at neutralizing the damaging effect. Examples of key players in that task are antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The second step involves an attempt to repair any damage caused to cellular constituents. An important role in that task is played by chaperons, which are active in unfolding denatured proteins. In case the repair system fails to reverse the cellular damage, the last step in the cellular response involves signaling and activation of processes responsible for cell death. The time-course of DJ-1 accumulation showing a 4 h lag following exposure to either H_2O_2 or MMC (Fig. 5), along with the result that less than an hour incubation is sufficient to induce apoptosis, shows that DJ-1 plays a delayed role in the cellular response to stress-inducing agents. Because neutralizing the damaging effect is likely to be activated rapidly, our results are consistent with a model in which DJ-1 plays a role in damage repair or in signaling the apoptotic pathway. Although our results cannot distinguish between these two potential functions, the latter is more favorable because of the demonstration of DJ-1 involvement in the control of apoptosis. On the other hand, DJ-1 has also been reported to have a chaperon-like activity [Lee et al., 2003], a finding that has not been corroborated [Olzmann et al., 2004].

In contrast to our findings, Yokota et al. [2003] have recently demonstrated that siRNA-mediated down-regulation of DJ-1 in the neuronal cell line Neuro2a did not sensitize the cells to the pro-apoptotic action of staurosporin. Staurosporin is a non-selective inhibitor of protein kinase C also known for its capacity to stimulate apoptosis in a variety of cells. The action of staurosporin was reported to be blocked in the neuroblastoma cell line SH-SY5Y by over-expression of the anti-apoptotic protein Bcl-X_L [Yuste et al., 2002]. Bcl-X_L is a member of the Bcl-2 family of proteins known to play a pivotal role in the regulation of mitochondrial integrity [Green and Reed, 1998; Wang and Reed, 1998] suggesting that staurosporin may act at the activation of the intrinsic pathway of apoptosis mediated by the release of several apoptogenic molecules from the intermembrane space of mitochondria. Because TRAIL activates the extrinsic pathway by interaction with specific death receptors, one attractive explanation for the difference between our results with TRAIL and those of Yokota et al. [2003] is that the activity of DJ-1 is directed at

steps that are unique to the extrinsic pathway. One potential step is at the level of TRAIL receptors where the efficiency of death signaling depends on the relative abundance of DR4 and DR5 to that of several decoy receptors [Ashkenazi and Dixit, 1999]. Indeed, up-regulation of a decoy receptor (DcR1) as a result of over-expression of the transcription factors Rel/NF-κB was shown to inhibit TRAIL signaling [Bernard et al., 2001]. In contrast, Nimmanapalli et al. [2001] showed that pretreatment of prostate cells with paclitaxel enhances TRAIL activity due to increase in the levels of DR4 and DR5.

A second potential site for DJ-1 action is the formation of the death-inducing signaling complex (DISC) [Kischkel et al., 1995] that includes the receptor itself, the adapter protein FADD and caspase-8 [Bodmer et al., 2000]. Formation of DISC triggers auto-processing and activation of caspase-8 [Medema et al., 1997] that in turn leads to the cleavage and activation of the effector caspase-3 or -7 [Srinivasula et al., 1996; Muzio et al., 1997]. DJ-1 may inhibit DISC formation and/or caspase-8 activation either directly or indirectly through a mechanism that involves cFLIP [Scaffidi et al., 1999], a protein that may have a negative dominant effect on caspase-8 [Srinivasula et al., 1997]. Activated caspase-8 may also cleave the proapoptotic BID protein, whose cleavage product triggers cytochrome c release from mitochondria [Li et al., 1998; Luo et al., 1998]. The mitochondrial step may be required to amplify the apoptotic signal and fully activate caspase-3 [Scaffidi et al., 1998]. In those cells where the release of proapoptotic molecules from the mitochondria is required for full activation of the apoptotic pathway, DJ-1 can potentially block BID cleavage and the transfer of the signal to the mitochondria. Such a mechanism was reported in LNCaP cells where PI 3-kinase-dependent arrest of TRAIL-induced apoptosis was mediated by Akt through the inhibition of BID cleavage [Nesterov et al., 2001].

PC-3 cells are known to have a mutant P53 and display absence of P53 nuclear immunostaining [Carroll et al., 1993; Rokhlin et al., 1997]. P53 plays a key role in the activation of the apoptotic pathway in many cells as a transcription factor that stimulates the transcription of many proapoptotic proteins [Levine, 1997] , as well as an activator of the intrinsic-mitochondrial pathway of apoptosis [Mihara et al., 2003]. Our demonstration that down-regulation of DJ-1 in PC-3 cells sensitizes the cells to the apoptotic-inducing action of TRAIL suggests that the role of DJ-1 in the control of apoptosis is independent of P53. The absence of P53 activity in PC-3 cells is unlikely to be responsible for the high level of DJ-1 expression, because a comparable level of DJ-1 appears in LNCaP cells (Fig. 1), a prostate cancer cell line that contains a functional P53 [Carroll et al., 1993].

The mechanism responsible for cellular resistance to TRAIL is obscure although inhibition in the capacity of the ligand to induce apoptosis has been demonstrated with several different cell lines [Griffith et al., 1998; Nesterov et al., 2001; Ng and Bonavida, 2002; Vivo et al., 2003], including PC-3 [Nimmanapalli et al., 2001; Sah et al., 2003]. In some cases, the action of TRAIL has been enhanced by concomitant incubation with one of several different agents including inhibitors of protein synthesis [Griffith et al., 1998; Yu et al., 2000; Chen et al., 2001; Munshi et al., 2001; Thakkar et al., 2001; Sah et al., 2003], actinomycin D—an inhibitor of RNA synthesis [Ng and Bonavida, 2002], paclitaxel—a drug that interferes with the normal recycling of the cytoskeletal tubulin [Nimmanapalli et al., 2001], or etoposide—a topoisomerase inhibitor [Vivo et al., 2003]. The mechanism for the synergistic activation is not yet known although activation of c-Jun N-terminal kinase (JNK) appeared to contribute to the cycloheximide-mediated induction of apoptosis by TRAIL [Sah et al., 2003; Vivo et al., 2003]. However, activation was only partial and it was suggested that suppression of other apoptosis-inhibitory activities or the activation of other signaling pathways must also be involved [Sah et al., 2003]. In addition to DJ-1 (the present study), suppression of glycogen synthase kinase 3β (GSK-3β) level in several human prostate cancer cell lines (including PC-3), sensitized the cells to the apoptotic action of TRAIL [Liao et al., 2003]. DJ-1 does not contain predicted GSK-3β phosphorylation sites, suggesting that these proteins either act independently in the TRAIL-control of cell death or that the interaction between DJ-1 and GSK-3β is indirect.

The mechanism by which DJ-1 regulates cell death appears complex. On one hand, a high level of DJ-1 expression renders cells resistant to TRAIL and other apoptotic-inducing agents whereas on the other hand, stimulation of the

level of DJ-1 appears to be associated with the execution of the apoptotic pathway in BPH-1 cells, cells that express an inducible form of DJ-1. The observation in which opposing effects may result in the same outcome is not without a precedent. For example, it was reported that both over-expression as well as decrease in the level of heat-shock proteins leads to growth inhibition in mammalian cells [Nollen and Morimoto, 2002]. Elucidation of the mechanism by which DJ-1 controls apoptosis in cells constitutively expressing a high level of the polypeptide and in cells expressing an inducible form of DJ-1 should assist in resolving the puzzle as to the true cellular role of DJ-1 and its mechanism of action.

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REFERENCES

Ashkenazi A, Dixit VM. 1998. Death receptors: Signaling and modulation. Science 281:1305–1308.

Ashkenazi A, Dixit VM. 1999. Apoptosis control by death and decoy receptors. Curr Opin Cell Biol 11:255–260.

Bernard D, Quatannens B, Vandenbunder B, Abbadie C. 2001. Rel/NF-kappaB transcription factors protect against tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by up-regulating the TRAIL decoy receptor DcR1. J Biol Chem 276: 27322–27328.

Bodmer JL, Holler N, Reynard S, Vinciguerra P, Schneider P, Juo P, Blenis J, Tschopp J. 2000. TRAIL receptor-2 signals apoptosis through FADD and caspase-8. Nat Cell Biol 2:241–243.

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. 2003. Mutations in the *DJ*-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256–259.

Brodie AE, Reed DJ. 1987. Reversible oxidation of glyceraldehyde 3-phosphate dehydrogenase thiols in human lung carcinoma cells by hydrogen peroxide. Biochem Biophys Res Commun 148:120–125.

Carroll AG, Voeller HJ, Sugars L, Gelmann EP. 1993. p53 oncogene mutations in three human prostate cancer cell lines. Prostate 23:123–134.

Chen X, Thakkar H, Tyan F, Gim S, Robinson H, Lee C, Pandey SK, Nwokorie C, Onwudiwe N, Srivastava RK. 2001. Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer. Oncogene 20: 6073–6083.

Cookson MR. 2003. Pathways to Parkinsonism. Neuron 37: 7–10.

Dawson TM, Dawson VL. 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science 302: 819–822.

El Ghalbzouri A, Hensbergen P, Gibbs S, Kempenaar J, van der Schors R, Ponec M. 2004. Fibroblasts facilitate re-epithelialization in wounded human skin equivalents. Lab Invest 84:102–112.

Goldstein S, Meyerstein D, Czapski G. 1993. The Fenton reagents. Free Radic Biol Med 15:435–445.

Green DR, Reed JC. 1998. Mitochondria and apoptosis. Science 281:1309–1312.

Griffith TS, Chin WA, Jackson GC, Lynch DH, Kubin MZ. 1998. Intracellular regulation of TRAIL-induced apoptosis in human melanoma cells. J Immunol 161:2833–2840.

Grzmil M, Voigt S, Thelen P, Hemmerlein B, Helmke K, Burfeind P. 2004. Up-regulated expression of the *MAT-8* gene in prostate cancer and its siRNA-mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cells. Int J Oncol 24:97–105.

Harlow E, Lane D. 1988. "Antibodies—A laboratory manual." Cold Spring harbor laboratory.

Hattori N, Kobayashi H, Sasaki-Hatano Y, Sato K, Mizuno Y. 2003. Familial Parkinson's disease: A hint to elucidate the mechanisms of nigral degeneration. J Neurol 250: 2–10.

Hayward SW, Dahiya R, Cunha GR, Bartek J, Deshpande N, Narayan P. 1995. Establishment and characterization of an immortalized but non-transformed human prostate epithelial cell line: BPH-1. In Vitro Cell Dev Biol Anim 31:14–24.

Hod Y, Pentyala SN, Whyard TC, El-Maghrabi MR. 1999. Identification and characterization of a novel protein that regulates RNA–protein interaction. J Cell Biochem 72: 435–444.

Honbou K, Suzuki NN, Horiuchi M, Niki T, Taira T, Ariga H, Inagaki F. 2003. The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease. J Biol Chem 278:31380–31384.

Huai Q, Sun Y, Wang H, Chin LS, Li L, Robinson H, Ke H. 2003. Crystal structure of DJ-1/RS and implication on familial Parkinson's disease. FEBS Lett 549:171–175.

Jenner P. 2003. Oxidative stress in Parkinson's disease. Ann Neurol 53(Suppl 3):S26–S36, discussion S36–S38.

Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME. 1995. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. Embo J 14:5579–5588.

Klinefelter GR, Welch JE, Perreault SD, Moore HD, Zucker RM, Suarez JD, Roberts NL, Bobseine K, Jeffay S. 2002. Localization of the sperm protein SP22 and inhibition of fertility in vivo and in vitro. J Androl 23: 48–63.

Le Naour F, Misek DE, Krause MC, Deneux L, Giordano TJ, Scholl S, Hanash SM. 2001. Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer. Clin Cancer Res 7:3328–3335.

Lee SJ, Kim SJ, Kim IK, Ko J, Jeong CS, Kim GH, Park C, Kang SO, Suh PG, Lee HS, Cha SS. 2003. Crystal structures of human DJ-1 and *Escherichia coli* Hsp31, which share an evolutionarily conserved domain. J Biol Chem 278:44552–44559.

Levine AJ. 1997. p53, the cellular gatekeeper for growth and division. Cell 88:323–331.

Li H, Zhu H, Xu CJ, Yuan J. 1998. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94:491–501.

Liao X, Zhang L, Thrasher JB, Du J, Li B. 2003. Glycogen synthase kinase-3beta suppression eliminates tumor necrosis factor-related apoptosis-inducing ligand resistance in prostate cancer. Mol Cancer Ther 2:1215–1222.

Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. 1998. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94:481–490.

Macedo MG, Anar B, Bronner IF, Cannella M, Squitieri F, Bonifati V, Hoogeveen A, Heutink P, Rizzu P. 2003. The DJ-1L166P mutant protein associated with early onset Parkinson's disease is unstable and forms higher-order protein complexes. Hum Mol Genet 12:2807–2816.

MacKeigan JP, Clements CM, Lich JD, Pope RM, Hod Y, Ting JP. 2003. Proteomic profiling drug-induced apoptosis in non-small cell lung carcinoma: Identification of RS/DJ-1 and RhoGDIalpha. Cancer Res 63:6928–6934.

Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME. 1997. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). Embo J 16:2794–2804.

Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll UM. 2003. p53 has a direct apoptogenic role at the mitochondria. Mol Cell 11:577–590.

Miller DW, Ahmad R, Hague S, Baptista MJ, Canet-Aviles R, McLendon C, Carter DM, Zhu PP, Stadler J, Chandran J, Klinefelter GR, Blackstone C, Cookson MR. 2003. L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system. J Biol Chem 278:36588–36595.

Mitsumoto A, Nakagawa Y. 2001. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. Free Radic Res 35:885–893.

Mitsumoto A, Nakagawa Y, Takeuchi A, Okawa K, Iwamatsu A, Takanezawa Y. 2001. Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat. Free Radic Res 35:301–310.

Munshi A, Pappas G, Honda T, McDonnell TJ, Younes A, Li Y, Meyn RE. 2001. TRAIL (APO-2L) induces apoptosis in human prostate cancer cells that is inhibitable by Bcl-2. Oncogene 20:3757–3765.

Muzio M, Salvesen GS, Dixit VM. 1997. FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogens. J Biol Chem 272:2952–2956.

Nachaliel N, Jain D, Hod Y. 1993. A cAMP-regulated RNA-binding protein that interacts with phosphoenolpyruvate carboxykinase (GTP) mRNA. J Biol Chem 268:24203–24209.

Nagakubo D, Taira T, Kitaura H, Ikeda M, Tamai K, Iguchi-Ariga SM, Ariga H. 1997. *DJ*-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. Biochem Biophys Res Commun 231:509–513.

Nesterov A, Lu X, Johnson M, Miller GJ, Ivashchenko Y, Kraft AS. 2001. Elevated AKT activity protects the prostate cancer cell line LNCaP from TRAIL-induced apoptosis. J Biol Chem 276:10767–10774.

Ng CP, Bonavida B. 2002. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: Sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl (Smac/DIABLO). Mol Cancer Ther 1:1051–1058.

Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle M, Lazebnik YA, et al. 1995. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 376:37–43.

Niki T, Takahashi-Niki K, Taira T, Iguchi-Ariga SM, Ariga H. 2003. DJBP: A novel DJ-1-binding protein, negatively regulates the androgen receptor by recruiting histone deacetylase complex, and DJ-1 antagonizes this inhibition by abrogation of this complex. Mol Cancer Res 1:247–261.

Nimmanapalli R, Perkins CL, Orlando M, O'Bryan E, Nguyen D, Bhalla KN. 2001. Pretreatment with paclitaxel enhances apo-2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of prostate cancer cells by inducing death receptors 4 and 5 protein levels. Cancer Res 61:759–763.

Nollen EA, Morimoto RI. 2002. Chaperoning signaling pathways: Molecular chaperones as stress-sensing 'heat shock' proteins. J Cell Sci 115:2809–2816.

Okada M, Matsumoto K, Niki T, Taira T, Iguchi-Ariga SM, Ariga H. 2002. DJ-1, a target protein for an endocrine disrupter, participates in the fertilization in mice. Biol Pharm Bull 25:853–856.

Olanow CW, Tatton WG. 1999. Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci 22:123–144.

Oliver FJ, de la Rubia G, Rolli V, Ruiz-Ruiz MC, de Murcia G, Murcia JM. 1998. Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant. J Biol Chem 273:33533–33539.

Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q, Ke H, Levey AI, Li L, Chin LS. 2004. Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. J Biol Chem 279: 8506–8515.

Orrenius S, McConkey DJ, Bellomo G, Nicotera P. 1989. Role of Ca^{2+} in toxic cell killing. Trends Pharmacol Sci 10:281–285.

Rokhlin OW, Bishop GA, Hostager BS, Waldschmidt TJ, Sidorenko SP, Pavloff N, Kiefer MC, Umansky SR, Glover RA, Cohen MB. 1997. Fas-mediated apoptosis in human prostatic carcinoma cell lines. Cancer Res 57: 1758–1768.

Sah NK, Munshi A, Kurland JF, McDonnell TJ, Su B, Meyn RE. 2003. Translation inhibitors sensitize prostate cancer cells to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by activating c-Jun N-terminal kinase. J Biol Chem 278: 20593–20602.

Sartorelli AC, Tomasz M, Rockwell S. 1993. Studies on the mechanism of the cytotoxic action of the mitomycin antibiotics in hypoxic and oxygenated EMT6 cells. Adv Enzyme Regul 33:3–17.

Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME. 1998. Two CD95 (APO-1/Fas) signaling pathways. Embo J 17:1675–1687.

Scaffidi C, Schmitz I, Krammer PH, Peter ME. 1999. The role of c-FLIP in modulation of CD95-induced apoptosis. J Biol Chem 274:1541–1548.

Spencer JP, Jenner A, Chimel K, Aruoma OI, Cross CE, Wu R, Halliwell B. 1995. DNA strand breakage and base modification induced by hydrogen peroxide treatment of human respiratory tract epithelial cells. FEBS Lett 374: 233–236.

Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Litwack G, Alnemri ES. 1996. Molecular ordering of the Fas-apoptotic pathway: The Fas/APO-1 protease Mch5 is a CrmA-inhibitable protease that activates multiple Ced-3/ICE-like cysteine proteases. Proc Natl Acad Sci USA 93: 14486–14491.

Srinivasula SM, Ahmad M, Ottilie S, Bullrich F, Banks S, Wang Y, Fernandes-Alnemri T, Croce CM, Litwack G, Tomaselli KJ, Armstrong RC, Alnemri ES. 1997. FLAME-1, a novel FADD-like anti-apoptotic molecule that regulates Fas/TNFR1-induced apoptosis. J Biol Chem 272:18542–18545.

Suliman A, Lam A, Datta R, Srivastava RK. 2001. Intracellular mechanisms of TRAIL: Apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 20:2122–2133.

Taira T, Saito Y, Niki T, Iguchi-Ariga SM, Takahashi K, Ariga H. 2004. DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep 5:213–218.

Takahashi K, Taira T, Niki T, Seino C, Iguchi-Ariga SM, Ariga H. 2001. DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. J Biol Chem 276:37556–37563.

Tao X, Tong L. 2003. Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease. J Biol Chem 278:31372–31379.

Thakkar H, Chen X, Tyan F, Gim S, Robinson H, Lee C, Pandey SK, Nwokorie C, Onwudiwe N, Srivastava RK. 2001. Pro-survival function of Akt/protein kinase B in prostate cancer cells. Relationship with TRAIL resistance. J Biol Chem 276:38361–38369.

Tomasz M, Lipman R, Chowdary D, Pawlak J, Verdine GL, Nakanishi K. 1987. Isolation and structure of a covalent cross-link adduct between mitomycin C and DNA. Science 235:1204–1208.

Vivo C, Liu W, Broaddus VC. 2003. c-Jun N-terminal kinase contributes to apoptotic synergy induced by tumor necrosis factor-related apoptosis-inducing ligand plus DNA damage in chemoresistant, p53 inactive mesothelioma cells. J Biol Chem 278:25461–25467.

Wagenfeld A, Yeung CH, Shivaji S, Sundareswaran VR, Ariga H, Cooper TG. 2000. Expression and cellular localization of contraception-associated protein. J Androl 21:954–963.

Wang HG, Reed JC. 1998. Mechanisms of Bcl-2 protein function. Histol Histopathol 13:521–530.

Welch JE, Barbee RR, Roberts NL, Suarez JD, Klinefelter GR. 1998. SP22: A novel fertility protein from a highly conserved gene family. J Androl 19:385–393.

Whyard TC, Cheung W, Sheynkin Y, Waltzer WC, Hod Y. 2000. Identification of RS as a flagellar and head sperm protein. Mol Reprod Dev 55:189–196.

Wilson MA, Collins JL, Hod Y, Ringe D, Petsko GA. 2003. The 1.1-A resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease. Proc Natl Acad Sci USA 100:9256–9261.

Wolf BB, Green DR. 1999. Suicidal tendencies: Apoptotic cell death by caspase family proteinases. J Biol Chem 274:20049–20052.

Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H. 2003. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem Biophys Res Commun 312:1342–1348.

Yoshida K, Sato Y, Yoshiike M, Nozawa S, Ariga H, Iwamoto T. 2003. Immunocytochemical localization of DJ-1 in human male reproductive tissue. Mol Reprod Dev 66:391–397.

Yu R, Mandlekar S, Ruben S, Ni J, Kong AN. 2000. Tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in androgen-independent prostate cancer cells. Cancer Res 60:2384–2389.

Yuste VJ, Sanchez-Lopez I, Sole C, Encinas M, Bayascas JR, Boix J, Comella JX. 2002. The prevention of the staurosporine-induced apoptosis by Bcl-X(L), but not by Bcl-2 or caspase inhibitors, allows the extensive differentiation of human neuroblastoma cells. J Neurochem 80:126–139.