DJ-1 Binds Androgen Receptor Directly and Mediates Its Activity in Hormonally Treated Prostate Cancer Cells

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Abstract

The oncogene DJ-1 has been associated with multiple cancers, including prostate cancer, where it can be stabilized by androgens and antiandrogens. However, little data exist on the expression pattern and function of DJ-1 in prostate cancer. To address the function of DJ-1 in prostate, a yeast two-hybrid screen was done to identify novel DJ-1 binding proteins. The androgen receptor (AR) was identified and confirmed as a DJ-1 binding partner. This is the first evidence that DJ-1 directly interacts with AR. We also show that modulation of DJ-1 expression regulated AR transcriptional activity. Importantly, both the subcellular localization of DJ-1 and the interaction with AR are regulated by androgens and antiandrogens. Additionally, immunohistochemical staining on two human prostate cancer tissue arrays was done providing the first large-scale expression analysis of DJ-1 in prostate. DJ-1 expression did not change with Gleason pattern but increased after androgen deprivation therapy, indicating that it may be involved in the development of androgen independence. These data provide a novel mechanism where DJ-1-mediated regulation of AR may promote the progression of prostate cancer to androgen independence. [Cancer Res 2007;67(10):4630–7]

Introduction

Multiple molecular events are involved in prostate cancer initiation, growth, invasion, and metastasis. Despite the diverse etiology, there is at least one universal similarity to the disease—the requirement of androgens and androgen receptor (AR) for tumor progression (1–7). Androgen deprivation therapy (ADT; also known as neoadjuvant hormone therapy) is initially effective to treat prostate cancer and remains the most common treatment regimen for advanced disease. Although ADT initially causes tumor regression, the eventual evolution of prostate cancer from an androgen-dependent to an androgen-independent phenotype allows continuation of tumor progression (reviewed in ref. 8). There are many hypotheses for the development of androgen-independent prostate cancer (AIPC). Some of these involve increased cellular proliferation/decreased apoptosis and, in some patients, increased serum prostate-specific antigen corresponding with promiscuous or increased AR activity (9, 10).

We hypothesized that identification of proteins that increase during ADT would modulate molecular changes that promote and/or maintain AIPC. To this end, a proteomic analysis of primary human prostate epithelial (HPE) cells after treatment with the antiandrogen flutamide was done (11). DJ-1 was identified as one protein that increased during flutamide treatment. Western blot analysis confirmed this result, showing that DJ-1 and AR protein levels increased following treatment with dihydrotestosterone (DHT), the synthetic androgen R1881, or the antiandrogen hydroxy-flutamide (OH-flutamide) in LNCaP cells. Blocking protein synthesis with cycloheximide revealed that the increase in DJ-1 was due to increased protein stability (11). This indicated that DJ-1 and AR expression was regulated by both androgens and antiandrogen therapy and may be involved in AIPC.

DJ-1 is a 20-kDa ubiquitous cytoplasmic and nuclear oncogene also known as PARK7 (12–14). Increased DJ-1 expression is capable of transforming NIH 3T3 cells either alone or, to a greater extent, in conjunction with c-myc or H-ras overexpression (15). Since this initial discovery, overexpression of DJ-1 has been associated with a number of human diseases including Parkinson’s disease (12, 14) and carcinomas of lung, breast, and prostate (16, 17). Increased DJ-1 expression in lung cancer correlates with poor clinical prognosis. Further, increased expression of DJ-1 in breast cancer has been associated with decreased expression of the tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN; ref. 17). Despite the oncogenic properties of DJ-1, its functional significance in prostate cancer development and progression has not been determined.

Previously, we showed that antiandrogen treatment could stimulate AR-regulated signaling in a subpopulation of HPE cells cultured from prostate biopsy specimens (11). Proteomic analysis identified DJ-1 as a protein whose expression was up-regulated by both androgen and antiandrogen treatment and that this increase was due to DJ-1 and AR protein stability. Therefore, identification of DJ-1 binding proteins would help elucidate the DJ-1 pathway in prostate cancer and its role in AIPC. To address this, primary HPE cells were derived from a punch biopsy of human prostate and the androgen responsiveness of these cells was determined using the transiently infected gene reporter assay as described (11). Briefly, this assay uses an androgen-responsive reporter to determine whether AR-regulated signaling in primary HPE cells is androgen responsive, androgen independent, or flutamide activated. Because we previously showed that DJ-1 increased after flutamide treatment, flutamide-activated HPE cells were used to generate a novel cDNA library. This library was screened using DJ-1 and identified AR as a DJ-1 binding partner. Previously, DJ-1 was thought to control AR activity through an indirect mechanism, although these studies were done after transient transfection of AR and DJ-1 in nonprostate cell lines (18, 19). This is the first evidence that endogenous DJ-1 directly binds AR. Additionally, DJ-1 subcellular localization and AR binding are hormonally regulated.
Further, two prostate cancer tissue arrays were analyzed, which provided the first large-scale expression analysis for DJ-I in prostate cancer. The first of the two arrays is arranged by Gleason pattern, and the second is arranged by length of time of ADT. DJ-I immunohistochemical staining on these arrays shows that DJ-I is expressed in benign as well as high Gleason pattern regions. DJ-I expression increased significantly in patients receiving >6 months of ADT, indicating that DJ-I may contribute to the development of AIPC. Taken together, these data provide a new mechanism for regulation of AR in androgen-independent prostate epithelial cells.

Materials and Methods

Yeast two-hybrid assay. Yeast two-hybrid assay was done using the Matchmaker System (Clontech) following the manufacturer's protocols. A cDNA library was generated from primary HPE cells that exhibited AR via activation via TIGR assay (11). cDNAs were cloned into the pGADT7 plasmid (Clontech) where they were expressed as fusion proteins to the GAL4 activation domain. For the bait protein, full-length human LNCaP cDNA was cloned into the pGBK7 plasmid and expressed as a fusion to GAL4 DNA binding domain. Plasmids were transformed into AH109 yeast strain and positive clones were selected on high-stringency dropout plates (−Leu/−Trp/−His/−Ade) containing X-a-galactoside. Plasmid DNA was extracted, transformed into DHS-α competent E. coli, and sequenced using T7 primer.

Cell culture and transfection. The LNCaP human prostate cancer cell line was obtained from American Type Culture Collection and was cultured as described (11). The LAPC4 human prostate cancer cell line was a gift from Dr. Charles L. Sawyers. LAPC4 cells were cultured in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Inc.) containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 10% charcoal-stripped FBS. On the third day, medium was again replaced with fresh medium containing 10% charcoal-stripped FBS and the following treatments: ethanol (vehicle control), 10−8 mol/L DHT (LNCaP), 10−9 mol/L DHT (LAPC4), 10−5 mol/L OH-flutamide (LNCaP), 10−6 mol/L OH-flutamide (LAPC4), 10−5 mol/L bicalutamide (LNCaP), or 10−5 mol/L bicalutamide (LAPC4). Optimal concentrations of androgens and antiandrogens for LNCaP cells were experimentally determined previously (11) whereas concentrations for LAPC4 cells were communicated from Dr. Sawyers' lab. Twenty-four hours after treatment, cells were harvested by trypsinization, and cytoplasmic and nuclear fractions were prepared using NEPER Kit and HALT protease inhibitors according to the manufacturer's protocol (Pierce Biotechnology).

Immunofluorescence and confocal microscopy. LNCaP and LAPC4 cells were grown on glass chamber slides (Lab-Tek Products). Cells were treated as described in nuclear/cytoplasmic extract section. Twenty-four hours after treatment, slides were fixed in 4% paraformaldehyde/PBS for 15 min, washed in PBS, permeabilized in PBS + 0.1% Triton X-100 for 5 min, and blocked in PBS with 3% BSA and 3% donkey serum for 30 min at room temperature. Slides were incubated with 1:1,000 dilution anti-AR; 1:500 dilution of mouse anti–SC-35 (Sigma) antibody overnight at 4°C, and subsequently incubated with Alexa Fluor 594–conjugated donkey anti-rabbit, Alexa Fluor 488–conjugated donkey anti-goat, and Alexa Fluor 647–conjugated donkey anti-mouse secondary antibodies (Molecular Probes) for 1 h at room temperature. The slides were washed, mounted in 50% glycerol, and imaged with a Zeiss LSM510 Meta Laser Scanning microscope. Stacks were acquired with LSM510 software; Z-projections and images were made with NIH ImageJ. All confocal images were acquired with a Plan-Apochromat 63×/1.4 oil differential interference contrast microscope objective at the following settings: wavelength 488 nm 5%, 543 nm 37%, and 633 nm 10%; filters Ch2-1; BP 505-550, Ch3-2; BP 560-615, and ChSi-3: 649-756 with a pinhole of 281 micrometers. Images have identical microscope settings between treatment groups. Image brightness/contrast was not altered between treatment groups; thus, intensity comparisons could be made. Quantification and images of colocalization were obtained using MetaMorph software.

Human tissue arrays and immunohistochemistry. Tissue microarrays (TMA) were generated using H&E slides from 112 radical prostatectomy specimens (from 1989 to 2003) obtained from the Vancouver General Hospital. Benign and cancer sites were identified and marked in donor paraffin blocks using matching H&E reference slides. TMA was constructed using a manual tissue microarrayer (Becker Instruments). Each marked block for benign and cancer was sampled four times with a core diameter of 0.6 mm arrayed in rectangular pattern with 1 mm between the centers of each core creating a quadruplicate TMA layout. Samples were ordered by histopathology and tumor Gleason grade or by length of ADT. The Gleason TMA contains 336 tissue cores representing 84 patients. ADT patients received monotherapy or combination therapy with the luteinizing hormone–releasing hormone antagonists Lupon or Zoladex and the antiandrogens flutamide or cyproterone acetate. The ADT TMA contains 294 cores representing 98 patients. DJ-I immunohistochemistry was done using 1:1,000 dilution of goat anti-DJ-I antibody (Abcam) and visualized using 3,3′-diaminobenzidine and standard techniques.

Hormones. DHT is commercially available from Sigma. OH-flutamide was purchased from LKT Laboratories, Inc. Bicalutamide was provided by AstraZeneca, Inc.

Plasmids and siRNA. DJ-I cDNA was cloned into pCruz-HA plasmid (Santa Cruz Biotechnology) and transiently transfected into LAPC4 cells using Lipofectamine 2000 (Invitrogen). Cells expressing NH2-terminally HA-tagged DJ-I were selected using 400 μg/mL G418. Western blot confirmed
expression of tagged DJ-1. To knock down DJ-1 expression, pooled siGENOME SMARTpool siRNAs (Dharmacon) directed against DJ-1 were transfected (200 pmol per well of a six-well plate) into LAPC4 cells using Lipofectamine 2000. Cells were harvested 48 h posttransfection. To ensure specificity of the chosen siRNA pool, a pool of nonspecific siRNAs (200 pmol per well of a six-well plate) was used as a control (Dharmacon Nontargeting siRNA #1).

**Results**

**DJ-1 binds the AR.** To identify DJ-1 binding partners, the yeast two-hybrid system and a cDNA library from flutamide-activated HPE cells were used. Full-length DJ-1 cDNA was used to screen the library and identified a region of AR (Fig. 1B). The AR fragment contained a region of the NH2-terminal activation domain, the entire DNA binding domain, and a portion of the ligand binding domain (Fig. 1A).

The DJ-1-AR interaction was confirmed by AR immunoprecipitation in the LAPC4 cell line. These androgen-dependent cells were derived from a lymph node metastasis and retain wild-type AR and prostate-specific antigen expression, unlike most prostate cancer cell lines that have lost AR expression or have AR mutations (21). Immunoprecipitation of endogenous AR pulled down DJ-1 (Fig. 1C). The reciprocal immunoprecipitation was done in LAPC4 cells expressing a NH2-terminally HA-tagged DJ-1. Immunoprecipitation with anti-HA antibody coimmunoprecipitated HA-tagged DJ-1, endogenous DJ-1, and AR. DJ-1 exists as an obligatory dimer (22). Therefore, we postulate that HA-DJ-1 binds endogenous DJ-1, explaining the precipitation of both tagged and untagged DJ-1 (Fig. 1D).

**Hormonal treatment increases the interaction between DJ-1 and AR.** Immunoprecipitations following hormonal treatment in LAPC4 and LNCaP cells were used to determine if these treatments regulated the DJ-1-AR interaction. LAPC4 and LNCaP cells were serum starved overnight and then treated with ethanol, 10^{-8} mol/L DHT (LNCaP), 10^{-9} mol/L DHT (LAPC4), 10^{-5} mol/L OH-flutamide (LNCaP), 10^{-6} mol/L OH-flutamide (LAPC4), 10^{-5} mol/L bicalutamide (LNCaP), or 10^{-6} mol/L bicalutamide (LAPC4) for 24 h. In both LAPC4 and LNCaP cells, antiandrogen treatment with either OH-flutamide or bicalutamide increased the amount of DJ-1 bound to AR (Fig. 2). DHT treatment seemed to increase the DJ-1-AR interaction in LNCaP cells more than in LAPC4 cells (Fig. 2).

**Androgens and antiandrogens increase DJ-1 nuclear localization.** To determine localization of DJ-1 in the prostate cancer cell lines LAPC4 and LNCaP, nuclear and cytoplasmic extracts were prepared. Cells were treated as described above. Twenty-four hours after treatment, cells were harvested and cytoplasmic and nuclear extracts were prepared using the NEPER kit (Pierce). Western blot analysis revealed that DJ-1 was predominantly cytoplasmic, but both androgen (DHT) and antiandrogen (OH-flutamide and bicalutamide) treatment increased DJ-1 expression in the nuclear fraction (Fig. 3A). As expected, AR expression was very low in the absence of androgens, but when treated with DHT, AR expression increased and was predominantly nuclear (Fig. 3A). Histone H1 and RhoA were used as nuclear and cytoplasmic controls, respectively, to show equal loading between lanes (Fig. 3A; ref. 23).

![Figure 1. AR binds DJ-1 in the yeast two-hybrid system and LAPC4 cell line. A, the fragment of AR identified spans the COOH-terminal region of the activation domain, the DNA binding domain, and a portion of the ligand binding domain. B, high-stringency selection showed that neither the bait (DJ-1) nor prey (AR) plasmid was capable of activating reporter genes individually, but the combination produced an interaction and activation of the reporters. C, immunoprecipitation (IP) of AR in LAPC4 cells pulled down DJ-1, confirming the interaction. D, reciprocal immunoprecipitation was done using LAPC4 cells that expressed HA-tagged DJ-1. Input lane contains total protein; mock immunoprecipitation was done with anti-IgG antibody to show specificity.](cancerres.aacrjournals.org/vol67/issue10/Figure1.jpg)

![Figure 2. Hormonal treatment increases DJ-1-AR interaction in LAPC4 and LNCaP cell lines. AR immunoprecipitation following treatment of LAPC4 and LNCaP cell lines with ethanol, DHT, OH-flutamide, and bicalutamide. Input lanes contain 5 μg of total protein lysate from DHT-treated sample. Mock immunoprecipitation was done with anti-IgG.](cancerres.aacrjournals.org/vol67/issue10/Figure2.jpg)
experiment was done in LNCaP cells, where an increase in DJ-1 after hormonal treatment was previously documented (11). LNCaP cells showed increased nuclear DJ-1 after hormonal treatment, with a greater increase in nuclear DJ-1 with DHT than in the LAPC4 cell line (Fig. 3B). AR was predominantly nuclear in all treatment groups, consistent with previous reports (24–26).

Immunofluorescence and confocal microscopy were used to confirm the presence of DJ-1 in both nuclear and cytoplasmic compartments. LAPC4 and LNCaP cells were plated on chamber slides and treated as described above. Cells were stained for DJ-1, AR, and SC-35 and visualized with appropriate secondary antibodies. SC-35 is a nuclear speckle marker and was included to identify sites of active transcription (27). As with nuclear and cytoplasmic extracts, DJ-1 (green) was predominantly cytoplasmic but was also present in the nucleus in both LAPC4 and LNCaP cell lines (Fig. 3C). AR (red) localization also confirmed results from nuclear and cytoplasmic extracts in that AR expression was low in the absence of androgens in LAPC4 cells but driven to the nucleus on treatment with DHT (Fig. 3C). Further, AR-positive nuclei were observed regardless of treatment in LNCaP cells, confirming observations from Western blot analyses (Fig. 3C). The merged images show colocalization between DJ-1 and AR, indicated by

**Figure 3.** Androgens and antiandrogens increase DJ-1 nuclear localization in LAPC4 and LNCaP cells. Nuclear and cytoplasmic extracts were prepared from LAPC4 cells (A) and LNCaP cells (B) after the indicated treatments. C, immunofluorescence for DJ-1 (green), AR (red), and SC-35 (blue) in LAPC4 and LNCaP cells following treatment. Merged high-magnification images show colocalization between DJ-1 and AR (yellow spots, white arrows). Colocalization between AR and SC-35 (pink/purple spots, white asterisk) marks active sites of transcription. Bar, 5 μm. D, images and quantification of colocalization between DJ-1 and AR. Yellow pixels, colocalization between DJ-1 and AR in LNCaP cells. White dashed lines outline several nuclei. Columns, mean percent of pixels that colocalized between DJ-1 and AR channels, determined in multiple optical slices for each treatment in LNCaP cells; bars, SE. Bar, 5 μm.
yellow in merged images (Fig. 3C, white arrows) but these areas appear distinct from nuclear speckles (Fig. 3C, white asterisks). Inset areas show low-magnification images of individual channels (Fig. 3C).

Yellow pixels from the LNCaP images in Fig. 3C were displayed alone and indicate that DJ-1-AR colocalization increased during hormonal treatment (Fig. 3D). White dashed lines outline nuclei (Fig. 3D). Colocalization of DJ-1 and AR was quantified by determining the percentage of colocalized pixels per image (Fig. 3D). DJ-1-AR colocalization was greatest with DHT treatment, followed by OH-flutamide and bicalutamide. Each treatment showed a distinct pattern. DHT and bicalutamide treatment resulted in similar colocalization throughout the nucleus, whereas DJ-1-AR with OH-flutamide treatment seemed to concentrate just within the nuclear perimeter. DJ-1-AR colocalization was not quantified in LAPC4 cells due to low AR levels except after DHT treatment.

DJ-1 regulates AR transcriptional activity. An androgen-responsive luciferase reporter was used to determine how modulation of DJ-1 expression affected AR activity. A region of the probasin promoter, termed (ARR2PB), containing two androgen responsive regions, was cloned upstream of the firefly luciferase gene resulting in the ARR2PB-luciferase reporter (11, 20). Overexpression of DJ-1 (HA-DJ-1 + luc) increased AR activity ∼20-fold (P < 0.001) compared with parental LAPC4 cells transfected with the luciferase reporter and with cells transfected with reporter and an empty plasmid control (Fig. 4A). Western blot analysis showed that transfection with pCruz-HA-DJ-1 plasmid doubled the expression level of DJ-1 but did not change the expression level of AR (Fig. 4B).

Similarly to overexpression, knockdown of DJ-1 expression influences AR-regulated transcription. Pooled siRNAs were cotransfected into LAPC4 cells along with the ARR2PB-luciferase reporter. Knockdown of DJ-1 expression decreased AR activity 5- to 10-fold compared with LAPC4 cells with reporter alone (P < 0.01; Fig. 4C). The decrease in DJ-1 expression after transfection of siRNAs resulted in ∼50% decrease in DJ-1 protein level compared with GAPDH loading control (Fig. 4D). Transfection of control nonspecific siRNA did not change DJ-1 expression compared with nontransfected LAPC4 cells. Knockdown of DJ-1 did not change AR expression, indicating that AR expression was not regulated by DJ-1 (Fig. 4D).

DJ-1 expression does not increase with Gleason pattern. Prostate samples from patients who underwent radical prostatectomy between 1989 and 2003 were obtained from Vancouver General Hospital. These patients received no treatment before surgery. The samples were grouped based on Gleason score and arranged on the slide based on predominant Gleason pattern for each tissue core in the following groups: benign, Gleason 2, Gleason 3, Gleason 4, and Gleason 5. The total number of patients represented by each group is shown (Fig. 5A).

DJ-1 immunohistochemical staining was done on this array and quantitated based on intensity. Four areas per tissue core were evaluated using the following scale: 0, no staining of any cells; 1, faint staining; 2, moderate intensity staining; and 3, intense staining. DJ-1 was present in all five sample groups at moderate intensity, but no statistically significant change was observed, indicating that DJ-1 does not increase with Gleason pattern (Fig. 5B). Figure 5C shows representative areas from each group at low and high magnifications (bars, 20 μm). DJ-1 is predominantly expressed in luminal epithelial cells and is both cytoplasmic and nuclear.

DJ-1 expression increases in human prostates with ADT. The second tissue array was generated identically to the first. This array was composed of samples from patients who received no ADT, <3 months ADT, 3 to 6 months ADT, or >6 months ADT before
undergoing radical prostatectomy surgery at Vancouver General Hospital. Figure 6A shows the number of patients per group.

DJ-1 staining intensity was evaluated identically to Gleason TMA. DJ-1 expression increased significantly in the >6 months ADT group as compared with the untreated group (P < 0.01; Fig. 6B). Figure 6C shows representative cores from each treatment group at low and high magnifications (bars, 20 μm). DJ-1 was both cytoplasmic and nuclear in luminal epithelial cells. The increase in the intensity of DJ-1 staining after prolonged ADT treatment suggests that DJ-1 may be involved in the emergence of AIPC as our previous data suggested.

Discussion

DJ-1 is a diverse signaling protein that seems to have multiple roles that may largely depend on cell type and cellular environment (13, 15–17, 19, 28, 29). DJ-1 was initially thought to activate AR through indirect mechanisms [e.g., by interacting with the AR binding region of protein inhibitor of activated STAT-α (PIASx-α) and preventing it from binding and inhibiting AR; ref. 19]. It has also been reported that DJ-1 represses AR activity in the SK-N- BE(2)C neuroblastoma cell line (30). From these conflicting reports, it seems that DJ-1 regulation of AR may be cell type dependent. We show that DJ-1 directly interacts with AR and functions as a positive regulator of AR signaling in two prostate cancer cell lines, possibly through multiple mechanisms.

Direct interaction between DJ-1 and AR was determined using a yeast two-hybrid screen of a cDNA library generated from flutamide-activated HPE cells (Fig. 1). Multiple yeast two-hybrid screens have been done to identify DJ-1 binding partners, but AR has never been reported among them (18, 19, 28, 29, 31). DJ-1 was shown to interact with the apoptotic protein Daxx when a human brain cDNA library was screened using this technique. This interaction was confirmed and shown to prevent cellular apoptosis following apoptotic stimuli in neuronal cells (28). The current screen did not identify previously identified DJ-1 binding partners, which may be due to differences in the cDNA libraries and cell lines used. Previous studies were done in COS and 293 cells and relied on transient transfection into these AR-negative cell lines, whereas our experiments were carried out with prostate cell lines and largely examined endogenous proteins.

The DJ-1-AR interaction is hormonally regulated in both LNCaP and LAPC4 cell lines. Specifically, antiandrogen treatment increased the DJ-1-AR interaction in both cell lines to a greater extent than DHT (Fig. 2). Immunofluorescence also showed that hormonal treatment increased the colocalization between DJ-1 and AR in LNCaP cells (Fig. 3C and D). Colocalization was not quantified from immunofluorescent images in LAPC4 cells due to low AR levels except after DHT treatment.

Further, both androgens and antiandrogens increased nuclear localization of DJ-1, providing a potential mechanism for DJ-1 regulation of AR in the nucleus. Increased nuclear DJ-1 was observed after androgen and antiandrogen treatment in both LAPC4 and LNCaP cell lines (Fig. 3A–C). In LAPC4 cells, DHT, OH-flutamide, and bicalutamide treatment resulted in approximately equal levels of nuclear DJ-1 (Fig. 3A). However, in LNCaP cells, DHT treatment resulted in higher levels of nuclear DJ-1 than either OH-flutamide or bicalutamide (Fig. 3B–D). Additionally, DHT and bicalutamide treatment resulted in DJ-1-AR colocalization throughout the nucleus, whereas DJ-1-AR seemed to concentrate just inside the nuclear perimeter after OH-flutamide treatment. These differences may be due to the well-characterized AR T877A substitution mutation in LNCaP cells (32–34). This mutation allows flutamide to exhibit agonist activity; however, because this mutation causes an AR conformational change, it could inhibit the interaction with DJ-1. Alternatively, the differences between the LNCaP and LAPC4 cells may be due to
differences in signaling pathways between the cell lines. For example, DJ-1 has been shown to antagonize the tumor suppressor PTEN (17). LNCaP cells lack functional PTEN (35) whereas the PTEN pathway is intact in LAPC4 cells (36). Because the exact role of DJ-1 in the PTEN pathway remains unclear, it is possible that loss or inactivation of members of this pathway alters the function of DJ-1.

Modulation of DJ-1 expression regulated AR transcriptional activity in LAPC4 cells, suggesting that overexpression of DJ-1 could facilitate AR activation in the absence of androgens (Fig. 4A and C). We previously identified DJ-1 as a protein that was up-regulated with antiandrogen treatment in primary HPE and LNCaP cells. TMA analysis of DJ-1 expression shows that DJ-1 did not change with Gleason pattern but increased significantly after 6 months of ADT (Figs. 5 and 6). This correlates with previous data and is the first reported large-scale evaluation of DJ-1 expression in prostate cancer. Additionally, the importance of this study should be emphasized due to the difficulty in obtaining tissue samples from patients who received ADT before surgery. However, the current study did not evaluate DJ-1 staining intensity in relation to the patient’s overall prostate cancer risk, which is determined by clinical variables such as prostate-specific antigen and total Gleason score. This is a potential future direction and will determine whether DJ-1 staining intensity correlates with prostate cancer recurrence or time to recurrence.

Current and previous data show that antiandrogens stabilize DJ-1, increase nuclear localization, and increase the interaction between DJ-1 and AR. Further, DJ-1 expression increases in patients who received >6 months ADT providing clinical evidence that DJ-1 may participate in AIPC. Taken together, these data show that DJ-1 is a novel AR binding protein that may be part of a class of coregulators modulated by antiandrogens. Factors that are regulated by antiandrogens, such as DJ-1, may increase during antiandrogen therapy to activate AR in the absence of androgens. Future studies are needed to identify other antiandrogen-regulated genes and to better understand the role they play in prostate cancer progression and activation of AR signaling in the absence of ligand.

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