Regulation of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by DJ-1 in thyroid cancer cells

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Abstract

DJ-1, a cancer-associated protein protects cells from multiple toxic stresses. The expression of DJ-1 and its influence on thyroid cancer cell death has not been investigated so far. We analyzed DJ-1 expression in human thyroid carcinoma cell lines and the effect of DJ-1 on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis. DJ-1 was expressed in human thyroid carcinoma cell lines; small interfering RNA-mediated downregulation of its levels significantly sensitized thyroid carcinoma cells to TRAIL-induced apoptosis, whereas the forced exogenous expression of DJ-1 significantly suppressed cell death induced by TRAIL. We also report here that TRAIL-induced thyroid cancer cell apoptosis is mediated by oxidative stress and that DJ-1, a potent nutritional antioxidant, protects cancer cells from apoptosis at least in part by impeding the elevation of reactive oxygen species levels induced by TRAIL and impairing caspase-8 activation. Subsequently, we investigated DJ-1 expression in 52 normal and 74 primary thyroid carcinomas from patients of China Medical University. The protein was not detectable in the 52 specimens of normal thyroid, while 70 out of 74 analyzed carcinomas (33 out of 33 follicular, 17 out of 19 papillary, 12 out of 13 medullar, and 8 out of 9 anaplastic) were clearly positive for DJ-1 expression. Our data demonstrated that DJ-1 is specifically expressed in thyroid carcinomas and not in the normal thyroid tissue. In addition, the protein modulates the response to TRAIL-mediated apoptosis in human neoplastic thyroid cells, at least partially through its antioxidant property.

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Introduction

DJ-1 was originally cloned as a putative oncogene capable of transforming NIH3T3 cells weakly on its own and more potently in combination with other oncogenes such as c-myc or ras (Nagakubo et al. 1997). Later, it was found to encode the protein involved in male fertility in rats and other mammals and able to work as a regulatory subunit of an RNA-binding complex (Hod et al. 1999). DJ-1 has also been isolated as a gene associated with autosomal early-onset Parkinson’s disease (Bonifati et al. 2003). The exact function of DJ-1 is obscure as yet and several diverse cellular roles including implication in transcriptional regulation, fertilization, and oxidative stress have been ascribed to it (Cookson 2003, Hod 2004).

Several lines of evidence suggest that DJ-1 plays a role in human tumorigenesis. First, breast cancer patients have elevated levels of DJ-1 and anti-DJ-1 autoantibodies in the sera compared with healthy and non-breast cancer patients (Le Naour et al. 2001). Second, DJ-1 levels were increased in primary lung tumors and in prostate cancer, and its expression correlated negatively with clinical outcomes in non-small cell lung carcinoma patients (MacKeigan et al. 2003, Grzmil et al. 2004, Hod 2004, Kim et al. 2005). In addition, treatment of human lung cancer NCI-H157…
cells with paclitaxel and MEK inhibitor U0126 leads to a decrease in DJ-1 protein expression (MacKeigan et al. 2003). DJ-1 affects cell survival, in part, by modulating cellular signaling cascades such as the PI3K survival pathway by negatively regulating the function of the tumor suppressor gene PTEN and altering p53 activity (Kim et al. 2005, Shinbo et al. 2005). Several studies have previously shown that DJ-1 expression in cancer cell lines conveys protection against multiple stresses, including chemotherapy, oxidative stress, endoplasmic reticulum stress, and proteosome inhibition (MacKeigan et al. 2003, Yokota et al. 2003, Taira et al. 2004).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) induces apoptosis in a variety of neoplastic cells while displaying minimal or absent cytotoxicity to most normal cells. Therefore, TRAIL is now considered a promising target for the development of anticancer therapies. The trimeric TRAIL binds to death receptors 4 (DR4) and 5 (DR5), an event followed by the recruitment of cytosolic adapter molecules and pro-caspase-8 to the DRs, forming the death-inducing signaling complex (DISC; Bodmer et al. 2000). The activation of caspase-8 by TRAIL induces the translocation of other cytosolic pro-apoptotic proteins to the mitochondria, causing a dissipation of the mitochondrial membrane potential (Yamada et al. 1999). Consequently, mitochondria release reactive oxygen species (ROS) and pro-apoptotic proteins into the cytoplasm thus inducing cellular and DNA damage (Wang & El-Deiry 2003). The activation of pro-caspase-8 is believed to be dependent solely on proximity to other pro-caspase-8 units during recruitment to the DISC (Muzio et al. 1998). However, accumulating lines of evidence have now demonstrated that ROS can modulate the initiation of apoptotic signaling (Perez-Cruz et al. 2003, 2007). Specifically, it has been suggested that ROS is necessary to initiate TRAIL-mediated apoptosis in some carcinoma cells (Lee et al. 2002a,b, Perez-Cruz et al. 2007).

The expression of DJ-1 in thyroid tumors and its influence on thyroid cell apoptosis have not been investigated so far. In the present study, we reported that DJ-1 levels were elevated in thyroid neoplastic cells. We showed that while the small interfering RNA (siRNA)-mediated knockdown of DJ-1 sensitizes thyroid carcinoma cells to TRAIL-mediated apoptosis, DJ-1 overexpression prevented apoptosis in thyroid cancer cell lines, consistent with the characteristics of oncogenes. We also demonstrated that oxidative stress is implicated as TRAIL-induced thyroid carcinoma cell death and DJ-1 modulates TRAIL-induced apoptosis at least in part by regulating the intracellular ROS levels.

Materials and methods

Cell cultures and reagent

All the cell lines were maintained in RPMI 1640 (Sigma–Aldrich) supplemented with 10% fetal bovine serum (Sigma–Aldrich), unless stated otherwise. Primary thyroid cancer cells were prepared as described previously (Metaye et al. 2002). Seven thyroid cancer samples were used in the study. The histological examination of the adjacent paraffin-embedded tissue was made in every case to confirm the histology of thyroid samples. Human recombinant TRAIL was obtained from Calbiochem (San Diego, CA, USA). 4,5-dihydroxy-1,3-benzenedisulphonic acid (Tiron) and KP372-1 were purchased from Sigma–Aldrich and Echelon Biosciences Incorporation (Salt Lake City, UT, USA) respectively.

Detection of cell death

For cell death assays, the cells were washed twice in PBS and then stained with Annexin V-FITC (Biovision, Mountain View, CA, USA) and propidium iodide (PI, Sigma–Aldrich) according to the manufacturer’s instructions. After staining with Annexin V-FITC and PI, the samples were analyzed using fluorescence-activated cell scanner (FACScan) flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Caspase-8 activity assay

Caspase-8 activity was measured using the caspase-8 colorimetric assay kit (Clontech), following the manufacturer’s instructions.

Measurement of intracellular ROS levels

The average level of intracellular ROS in thyroid cancer cells was evaluated in cells loaded with the redox-sensitive dye CM-H2DCFDA (Molecular Probes, Eugene, OR, USA). The cells were washed twice in PBS, stained in darkness for 30 min with 20 μM CM-H2DCFDA, and harvested. The cells were dissolved with 1% Triton X-100, and fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength using a fluorescence spectrometer (HTS 7000; Perkin–Elmer, Boston, MA, USA). A duplicate culture with the same treatment was used to determine the total protein levels. The ROS levels were expressed as arbitrary units/mg protein.
Construction of DJ-1 plasmid and generation of FRO cells stably overexpressing DJ-1

A cDNA encoding human DJ-1 was generated by PCR from human brain cDNA library (Invitrogen) and subcloned into the EcoRI/XhoI sites of the eukaryotic expression plasmid pcDNA3 tagged with FLAG. The construct was verified by DNA sequencing. The cells were transfected with Lipofectamine 2000 reagent (Invitrogen) as instructed by the supplier. The control cells were transfected with the pcDNA3-FLAG. The transfected cells were selected in a medium containing G418 (800 μg/ml) and resistant colonies were expanded. Western analyses employing the antibodies to DJ-1 or FLAG were used to identify positively expressing cells.

Small interfering RNA (siRNA)
The siRNA sequences used here were as follows: siDJ-1, UGGAGACGGUCAUCCUGUTT (Taira et al. 2004, Clements et al. 2006). The scramble nonsense siRNA (scramble, CCGUAUCGUAAGCAGUACU), which has no homology to any known genes was used as the control. In addition, a position mismatched (sequence underlined) siDJ-1 (simutDJ-1; UGGAGACGGUCA-GACCUGUTT) was also used to confirm the specificity of siDJ-1. The transfection of the siRNA oligonucleotide was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendations. The cells were transfected on three consecutive days, and the lysates were taken for protein analysis 72 h after the first transfection.

Western blot analysis
The cells were lysed in a lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail (Sigma–Aldrich)). The cell extract protein amounts were quantified using the BSA protein assay kit. Equivalent amounts of protein (25 μg) were separated using 12 or 16% SDS-PAGE and transferred to the PVDF membrane (Millipore Corporation, Billerica, MA, USA). Western immunoblotting was performed using anti-human DJ-1 monoclonal antibody (Abcam, Cambridge, UK), anti-human DR4 polyclonal antibody (ProSci Incorporated, Poway, CA, USA), anti-human DR5 (Abcam), anti-human DcR1 polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), anti-human DcR2 polyclonal antibody (Imagenex, San Diego, CA, USA), FLAG (Sigma–Aldrich), or γ-tubulin (Sigma–Aldrich), horse-radish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (Amersham Biosciences), and ECL solutions (Amersham Biosciences).

Akt/PKB activity assay
The cells were homogenized as described above (see section on western blotting analysis) and Akt in the supernatants extracted using a procedure that involves incubation with an Akt antibody (Calbiochem) and adsorption of immunoprecipitated Akt onto Protein A/Protein G Plus agarose beads (Calbiochem). The Akt activity was measured using K-LISAAkt activity assay kit (Calbiochem) according to the manufacturer’s instructions, and the enzyme activity expressed as absorbance at 450 nm per 10 mg protein.

Immunohistochemistry
Specimens from normal and pathological human thyroid tissues were isolated, rinsed with PBS, fixed in 4% buffered neutral formalin, and embedded in paraffin. Using the standard avidin–biotin peroxidase method (Elite standard kit SK6100; Vector Laboratories, Burlingame, CA, USA), deparaffinized sections were stained with primary antibody against DJ-1. After incubation in a solution containing diaminobenzidine and hydrogen peroxide in PBS for 5 min, the slides were washed, dehydrated with alcohol and xylene, and mounted with cover slips using a permanent mounting medium.

Data analysis
Statistical difference was evaluated using the two-tailed Student’s t-test; P<0.05 was considered significant.

Results
Expression of DJ-1 in human thyroid cancer cell lines
We first analyzed the expression of DJ-1 in a panel of human thyroid tumor cell lines and found significant levels of the protein in some human thyroid carcinoma cell lines (Fig. 1A). It has been previously reported that DJ-1 is involved in the control of cell death upon exposure to TRAIL in prostate cell lines; cells expressing a low but inducible level of DJ-1 are sensitive to TRAIL-induced apoptosis, whereas those expressing a high but constitutive level of DJ-1 are resistant to TRAIL-induced apoptosis (Hod 2004). To clarify whether this is the case in the panel of thyroid carcinoma cells, the thyroid cancer cells were treated with TRAIL for 24 h, and the DJ-1 level (Fig. 1B) and
cell death (Fig. 1C) were evaluated subsequently. The expression level of DJ-1 appeared to be inversely correlated with TRAIL-induced apoptosis in this panel of thyroid carcinoma cells. FRO and KTC2 cells with low expression levels of DJ-1 demonstrated a high sensitivity to TRAIL-induced cell death. On the other hand, ARO, KTC1, KTC3, CGTHW-3, FTC-133, and TT cells with higher expression levels of DJ-1 revealed to be less sensitive to the same treatment (Fig. 1A–C). However, no obvious alteration in DJ-1 levels was detected with TRAIL treatment in any cell line, including FRO and KTC2 cells, which express little DJ-1 under basal conditions and reveal a high sensitivity to TRAIL-induced apoptosis (Fig. 1B and C). In the following studies, we selected ARO and FRO cells representing TRAIL-insensitive and -sensitive cells respectively.

Figure 1 Expression of DJ-1 in thyroid cancer cell lines. (A) Western blot analysis was performed in cell lysates derived from a panel of thyroid cancer cell lines. (B) Thyroid cancer cells were treated with 1000 ng/ml TRAIL (20 ng/ml for FRO cells) for 24 h and western blot analysis was performed. (C) The cells were treated as in (B) and apoptosis was measured. The experiments were repeated thrice and the data are presented as the mean ± S.D. of representative experiments performed in triplicate.

siRNA-mediated downregulation of DJ-1 sensitizes thyroid carcinoma cells to TRAIL

To investigate the impact of DJ-1 on the sensitivity of thyroid cancer cells toward TRAIL, we employed DJ-1-specific siRNA (siDJ-1) to test the consequence of the reduced DJ-1 expression on TRAIL-mediated induction of apoptosis in thyroid carcinoma cells. A scrambled siRNA as well as a site-mutated siRNA against DJ-1 (simutDJ-1) were utilized as controls. The cells transfected with siDJ-1, but not scramble or simutDJ-1, displayed a reduced level of DJ-1 in the immunoblotting of total cell protein lysates (Fig. 2A). Seventy-two hours after the first transfection, the cells were incubated in the presence of various doses of TRAIL for an additional 24 h after which apoptosis was evaluated. Under these conditions, apoptosis in thyroid carcinoma cells transfected with siDJ-1 was accelerated when compared with that of non-transfected cells and that of cells transfected with simutDJ-1 or scrambled siRNA (Fig. 2B). While TRAIL significantly induced apoptosis in siDJ-1-transfected ARO cells incubated with a concentration as low as 500–1000 ng/ml, no cell death was observed in scramble or simutDJ-1-transfected cells even at a concentration as high as 2000 ng/ml (Fig. 2B).

We also asked whether the downregulation of DJ-1 may further sensitize FRO cells to TRAIL-induced cell death and thus lower the concentration of TRAIL required for tumor cell kill. To this end, we knocked down DJ-1 in FRO cells (Fig. 2C) and analyzed TRAIL-induced cell death. Though less marked than that in ARO cells, the downregulation of DJ-1 also increased TRAIL-induced cytotoxicity in FRO cells (Fig. 2D). The downregulation of DJ-1 also sensitized other types of thyroid cancer cells such as CGTHW-3 (papillary), FTC-133 (follicular), and TT (medullary) (data not shown).

To clarify whether an alteration of DRs might be implicated in the sensitizing effect of siDJ-1, the levels of TRAIL receptors DR4 and DR5, as well as decoy receptors DcR1 and DcR2, were evaluated. As shown in Fig. 2A and C, siDJ-1 had no effect on the expression levels of these receptors, suggesting that DRs might not be involved in the sensitizing effect of DJ-1 downregulation.

Overexpression of DJ-1 inhibits TRAIL-induced apoptosis

To further link the expression of DJ-1 with the control of cell death, we established FRO cell lines harboring exogenously expressed FLAG-tagged DJ-1 (designated DJ-1/F), the transcription of its gene was driven...
by a cytomegalovirus (CMV) promoter. Western blot analysis of total cell lysates confirmed the overexpression of DJ-1-engineered cells when compared with parental or empty vector-transfected cells (Fig. 3A). It should be noted that the forced expression of DJ-1 had no effect on the expression level of TRAIL receptors DR4 and DR5, as well as decoy receptors DcR1 and DcR2 (Fig. 3A).

To determine whether the overexpression of DJ-1 affects the cell viability against TRAIL, viabilities of DJ-1/F#2 and DJ-1/F#9 stable cells, which demonstrated the highest expression level of exogenous DJ-1, in the presence of TRAIL were determined. Contrary to the case of DJ-1 knockdown cells, cells harboring exogenously overexpressed DJ-1 were much more resistant to TRAIL than parental non-transfected or FLAG stable cells. DJ-1/F cells had higher ID50 (50–100 ng/ml) when compared with parental and FLAG cells (5–10 ng/ml), suggesting that the high level of DJ-1 may confer cells with resistance to TRAIL (Fig. 3B). To further confirm whether the exogenously expressed DJ-1 accounted for the reduction of apoptosis induced by TRAIL, we investigated the effect of siDJ-1. The knockdown of DJ-1 levels recovered the responsiveness of DJ-1 stable cells to TRAIL-induced cell death, similar to that of parental or FLAG stable cells (Fig. 3C).

**DJ-1 modulates TRAIL-induced apoptosis by influencing intracellular ROS generation**

The generation of ROS is now considered to be the early and critical event for the initiation of TRAIL-induced apoptotic signaling in some human cancer cell lines (Lee et al. 2002a,b, Perez-Cruz et al. 2007). As DJ-1 functions as a potent antioxidant molecule, we speculated that DJ-1 might suppress apoptosis by impeding the generation of intracellular ROS induced by TRAIL. To clarify whether the increase in intracellular ROS is implicated in TRAIL-induced apoptosis in our panel of thyroid carcinoma cells, we first performed fluorescence spectrometry in these cells after loading them with CM-H2DCFDA, a dye that
becomes fluorescent upon oxidation with H$_2$O$_2$, hydroxyl radical, peroxyl radical, and peroxynitrite anion. We found that an increase in the amount of intracellular ROS appeared to be positively correlated with apoptosis induced by TRAIL. TRAIL rapidly increased the amount of intracellular ROS in TRAIL-sensitive FRO cells (Fig. 4A). By contrast, no alteration was observed in ARO cells, which poorly responded to TRAIL (Fig. 4A). In addition, TRAIL-induced ROS generation was dramatically reduced in the DJ-1/F FRO cell lines compared with parental FRO cells (Fig. 4B). On the other hand, the generation of ROS was markedly increased in the siDJ-1-transfected cells compared with cells transfected with scramble siRNA upon exposure to TRAIL (Fig. 4C). To examine whether elevated ROS is crucial for the sensitizing effects of DJ-1 siRNA on TRAIL-induced apoptosis in ARO cells, the cell-permeable superoxide scavenger Tiron was utilized to perturb the TRAIL-induced ROS generation and then to examine the consequent events. Pretreatment with 1 mM Tiron for 4 h resulted in the complete restoration of TRAIL-induced generation of ROS (Fig. 4D). The abolishment of intracellular ROS by Tiron completely abrogated the activation of caspase-8 (Fig. 4E), as well as the apoptosis caused by TRAIL (Fig. 4F).

Since DJ-1 has also been shown to be implicated in the regulation of Akt signal (Kim et al. 2005, Yang et al. 2005, Hudson et al. 2007), to verify the role of Akt activity in the effect of DJ-1, the Akt activity was

Figure 3 Overexpression of DJ-1 partially protects FRO cells from TRAIL-induced cell death. (A) Cell lysates from parent FRO, FLAG stable or DJ-1 stable (DJ-1/F) cells were analyzed by western blot. (B) The cells were treated with the indicated amount of TRAIL for 24 h, and apoptosis was measured. The experiments were repeated thrice and the data are presented as the mean ± s.d. of representative experiments performed in triplicate. * $P<0.05$. (C) siRNA (simutDJ-1 or siDJ-1)-transfected DJ-1/F#2 and DJ-1/F#9 stable cells were treated with 20 ng/ml TRAIL for 24 h and cell death was analyzed. The experiments were repeated thrice and the data are presented as the mean ± s.d. of representative experiments performed in triplicate. **$P<0.01$; NS, not significant.
first measured in ARO and FRO cells (Fig. 4G). Consistent with previous reports, Akt activity appeared to be positively correlated with DJ-1 expression level: DJ-1 overexpression cells demonstrated increased Akt activity, whereas siDJ-1 significantly decreased Akt activity (Fig. 4G). The Akt inhibitor KP372-1 (50 nM) partially abrogated the DJ-1-mediated suppression of apoptosis induced by TRAIL (Fig. 4H); on the other hand, KP372-1 had no effect on the Tiron-mediated inhibition of TRAIL (Fig. 4H), indicating that Akt may in part be implicated in the action of DJ-1, and take an effect upstream of ROS generation.

**DJ-1 is upregulated in primary thyroid cancer**

To investigate the expression of DJ-1 in primary thyroid malignant lesions, tissue blocks from 74 primary thyroid cancer patients were analyzed by immunohistochemistry for DJ-1. Negative controls with preabsorbed primary antibody or without primary antibody had no signal (data not shown). Normal thyroid specimens were negative for DJ-1 expression (Fig. 5A). On the other hand, 70 out of 74 analyzed carcinoma specimens were clearly positive for DJ-1 expression (Fig. 5A). Similarly, 33 out of 33 papillary, 17 out of 19 follicular, 12 out of 13 medullary, and 8 out of 9 anaplastic carcinomas were positive for DJ-1. We further analyzed DJ-1 expression in fresh thyroid samples using western blot analysis (Fig. 5B). The normal or benign thyroid tissues demonstrated no or little expression of DJ-1, whereas most of malignant thyroid tissues highly expressed DJ-1 (Fig. 5B and C). A variation was observed among cases, whereas there was no significant difference among papillary, follicular, anaplastic, and medullary thyroid cancers (Fig. 5B and C). We further verified the possible implication of DJ-1 in the responsiveness to TRAIL in cultured primary thyroid cancer cells, and found that cells with a high DJ-1 expression demonstrated less sensitivity to TRAIL treatment compared with those with low DJ-1 expression (Fig. 5D).

**Discussion**

In the present study, we reported that DJ-1 modulated TRAIL-mediated apoptosis in thyroid cancer cell lines. This conclusion is supported by the demonstration that siRNA-mediated reduced expression of DJ-1 sensitized the otherwise resistant thyroid cancer cells to the same apoptotic-inducing ligand, as well as overexpression of DJ-1 desensitized the otherwise sensitive thyroid cancer cell lines by preventing the rise in...
intracellular ROS levels and the activation of caspase-8 induced by TRAIL.

TRAIL is a transmembrane protein which shares homology in its extracellular domains with other members of the TNF family of the apoptosis-inducing ligand. The ligand has been shown to trigger apoptosis in a wide variety of transformed and cancerous cells via the interaction with specific death domains containing receptors (DR4 and DR5). Upon activation, the DRs initiate a cascade pathway that leads to the proteolytic activation of the effector caspase-3. Oxidative stress is a complex and dynamic situation characterized by an imbalance between the production of ROS and the availability and action of antioxidant principles. Oxidative stress has traditionally been considered in negative terms due to its association with macromolecular damage and the induction of cytotoxicity. In this regard, the generation of ROS is now considered to be the early and critical event for the initiation of TRAIL-induced apoptotic signaling in some human cancer cell lines (Lee et al. 2002a,b, Evans et al. 2003, Meurette et al. 2005, Basu et al. 2006, Kwon & Choi 2006, Nagy et al. 2006, Perez-Cruz et al. 2007). Numerous studies have firmly established the protective role of DJ-1 in oxidative stress (Kinumi et al. 2004, Taira et al. 2004, Menzies et al. 2005, Ooe et al. 2005, Yang et al. 2005, Zhou & Freed 2005, Meulener et al. 2006). The potential role of DJ-1 in the control of cell death was suggested earlier by the demonstration that the induction of apoptosis in a non-small cell lung carcinoma resulted in a concomitant marked decline in the level of DJ-1 (MacKeigan et al. 2003). Interestingly, DJ-1 appeared to play a different role between cells expressing a low but inducible level of DJ-1 and those expressing a high but constitutive level of DJ-1 in prostate cell lines (Hod 2004). A high constitutive level of DJ-1 expression rendered cells resistant to TRAIL, whereas the upregulation of DJ-1 appeared to be associated with the induction of apoptosis in benign prostate cells expressing a low basal level of DJ-1 (Hod 2004). We also found that the basal level of DJ-1 appeared to be inversely correlated with TRAIL-induced apoptosis, whereas the upregulation of DJ-1 appeared to be associated with the induction of apoptosis in benign prostate cells expressing a low basal level of DJ-1 (Hod 2004). We also found that the basal level of DJ-1 appeared to be inversely correlated with TRAIL-induced apoptosis. However, we could not detect the induction of DJ-1 in any thyroid cancer cell lines investigated, including FRO and KTC2 cells, which expressed a very low basal level of DJ-1 and were sensitive to TRAIL-induced apoptosis compared with other cell lines.

Figure 5 The expression of DJ-1 in primary thyroid carcinoma tissues. (A) Representative DJ-1 staining images of thyroid tissues are presented. (B) Representative western blotting images from fresh thyroid tissues are presented. (C) Arbitrary units of DJ-1 (normalized by γ-tubulin) are blotted on the scatter diagram. (D) Primary thyroid cancer cells were treated with the TRAIL (1000 ng/ml) for 24 h, and apoptosis was measured. A representative image of DJ-1 expression is presented at the bottom of the histogram.
The different induction of DJ-1 by TRAIL in prostate and thyroid cells suggested that TRAIL-mediated induction of DJ-1 may be a cell type specific, but not a common phenomenon. Alternatively, DJ-1 might differently respond to TRAIL in benign and cancer cells. Taken together, these results strongly suggested that DJ-1 could be involved in the control of cell death in mammalian cells, and the mechanism underlying the regulation of cell death by DJ-1 appears complex.

One of the major mechanisms by which cells protect themselves against oxidative stress is the upregulation of a wide range of antioxidant genes. Among the intracellular antioxidant molecules, reduced glutathione (GSH) is the most abundant intracellular non-protein thiol in cells. It is deserved to note that DJ-1 increases GSH synthesis by upregulating transcription of glutamate cysteine ligase, a rate-limiting enzyme in GSH synthesis during oxidative stress (Zhou & Freed 2005). It has also been reported that DJ-1 increases GSH synthesis by upregulating transcription of glutamate cysteine ligase, a rate-limiting enzyme in GSH synthesis during oxidative stress (Zhou & Freed 2005). It has also been reported that DJ-1 stabilizes the antioxidant transcriptional master regulator Nrf2, which induces the expression of antioxidant genes (Clements et al. 2006). Many Nrf2 target genes could be beneficial to the survival of an existing tumor and have been linked to drug resistance (McLellan & Wolf 1999). Moreover, Nrf2 can be activated by certain anticancer agents, which will influence the effectiveness of chemotherapy (Jin et al. 2006). Thus, the enhanced expression of DJ-1 in cancer cells is likely to provide a survival advantage via the increase in detoxification enzymes, underscoring that the potential of these enzymes may be exploited as treatment target in tumors expressing high level of DJ-1.

Previous study has identified DJ-1 as a potential tumor antigen that is found in the circulation of breast tumor-bearing patients (Le Naour et al. 2001). This report raises the intriguing possibility that DJ-1 may be secreted or released into the serum, emphasizing its potential as a tumor marker. Our data indicate that DJ-1 is overexpressed in thyroid tumors. Whether DJ-1 is secreted into the circulation in thyroid tumor patients deserves to be clarified in future studies.

Our data show that the reduction of DJ-1 is associated with greater apoptotic cell death, whereas the introduction of DJ-1 into cells enhanced cell survival, consistent with a role for DJ-1 in the survival of tumors. The overexpressed protective proteins provide better potential drug targets for the development of small molecule therapeutics. Thus, using siRNA or small molecules to specifically target DJ-1 in combination with TRAIL could be a valuable approach to enhance treatment for thyroid tumor patients with high DJ-1 levels.

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