Original Article

DJ-1 inhibits the progression of papillary thyroid carcinoma by targeting akt pathway

Juyuan Bu1*, Guowei Li2*, Xiaoyu Xiao3, Yingxian Zhu3, Zibin Liang4, Bingzong Hou1

Departments of 1General Surgery I, 2Orthopedics II, 3Anesthesiology, 4Radiation Oncology, Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, Guangdong, China. *Co-first authors.

Received November 2, 2015; Accepted January 5, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Thyroid cancer is the most frequent type of malignant tumor in the endocrine system; DJ-1 has been recognized as a key player in thyroid cancer. In this study, we sought to explore the correlation of DJ-1 expression with cell proliferation, invasion and apoptosis in papillary thyroid cancer. To investigate the roles of DJ-1 in regulating the papillary thyroid cancer, we transfected si-DJ-1 into B-CPAP and CGTH-w3 cell lines, and then we tested the cell proliferation, invasion and apoptosis in papillary thyroid cancer by RT-PCR and western blot assay. The results indicated that the proliferation rate, invasive cell number and migration ability of papillary thyroid carcinoma cells, as well as Akt in the siDJ-1 transfected group significantly decreased compared with blank group. Moreover, proteins like MMP2 and MMP9 expression, was also affected by the DJ-1 expression changes. These results proved that DJ-1 could be involved in progression of papillary thyroid carcinoma by Akt signaling pathways. This study revealed the key roles of DJ-1 and its potential in preventing and treating papillary thyroid carcinoma.

Keywords: Thyroid cancer, DJ-1, akt pathway

Introduction

Thyroid cancer is one of the common malignant tumor in the endocrine system, taking up 1% of the whole body malignant tumor. Papillary thyroid carcinoma (PTC) is the most common pathological type occurring on head and neck, accounting for 60%~80% of all adults thyroid malignant tumor, and of all thyroid carcinoma in children [1-3]. PTC has also been reported to be associated with high recurrence and potentially increased mortality. Increased in recent years, the incidence of papillary thyroid carcinoma in our country, especially in the coastal city, revealed an average annual growth of 4%. Many factors had been found to be related to the initiation, development, and metastasis of PTC [1, 4, 5]. In the study of Simms A, et al., TROP-2 was found playing an important role in diagnosing classic PTC, especially in equivocal cases. In the other research, miR-29a expression was found notably associated with the PTC tumor size, the final research suggesting that miR-29a could act as a tumor suppressor in PTC and that miR-29a may potentially serve as an anti-tumor agent in the treatment of PTC. The expression and role of RR in thyroid carcinoma (TC) has also been deeply investigated [6-8]. While research on the PTC has lasted years, there remains no clear understanding of this kind of disease.

DJ-1 was discovered in 1997, its coding protein DJ-1 was proved to have the ability of cell transformation and exerts a protective role against oxidative stress damage, working as a cellular oxidative stress sensor, and it seems to regulate gene expression at different levels [9]. Also, DJ-1 is announced to be an endogenous antioxidant against cancer, neurodegeneration and cardiovascular diseases, of which oxidative stress plays a causal role, it follows that activation of DJ-1 as a common endogenous antioxidant provides a new strategy against these kind of diseases, including cancers [10-14]. So far, the existing related data displayed DJ-1 expression in breast cancer, laryngeal squamous and cervical cancer tissue. Previous studies demonstrated the importance of DJ-1 in the existence of breast cancer, thus, DJ-1 could serve as the...
Roles of DJ-1 in papillary thyroid carcinoma cell lines


Biological mechanism research on laryngeal squamous cell carcinoma has confirmed the association between DJ-1 and tumors [16-18]. Moreover, relevant researches tried to detect the expression of DJ-1 case in thyroid cancer clinical specimens, finding that its expression in thyroid carcinoma was higher than normal tissues. Elucidating the molecular biological mechanism in PTC may lead to the identification of the critical insight into the pathogenesis of PTC. So far, little is known regarding the specific role of DJ-1 gene in the thyroid papillary carcinoma and its mechanism research is still lacking.

In this study, we will preliminarily investigate the biological function and its possible mechanism of DJ-1 in thyroid papillary cancer cells. We investigated the proliferation, invasion and apoptosis of the PTC cell lines in vitro, and confirm the connection between the DJ-1 and thyroid papillary carcinoma. Besides, expression changes of MMP2 and MMP9 were proved to be infected by the expression of DJ-1 by western blot assay results. The present study provides a new understanding of the regulated mechanism of DJ-1 in PTC and identifies potential avenues for the therapeutic intervention for this disease.

Materials and methods

Cell culture

All the cell lines were maintained in RPMI 1640 (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Sigma-Aldrich), unless stated otherwise [19].

Real-time PCR

Total mRNA was isolated from cells and islets as previously described [20]. Complementary DNA (cDNA) was produced using reverse transcriptase (iScript™ cDNA Synthesis Kit; Bio-Rad Laboratories). The expression levels of mRNAs were measured by SYBR green-based quantitative RT-PCR (SYBR Green Master mix; Thermo Scientific, Waltham, MA, USA) [20].

Plasmids and siRNA transfection

The shRNA expressing plasmids specifically targeting DJ-1 and control siRNA (no silencing) were synthesized by GenePharma Co (Shanghai, China). A DJ-1 expression vector (pcDNA3.1-DJ-1) was constructed by sub-cloning the full-length wild-type DJ-1 coding sequence into pcDNA3.1(+), and confirmed by sequencing. The empty construct pcDNA3.1 was transfected as a control. Cell transfections were conducted using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol. Stable DJ-1 transfectants were generated under G418 (Gibco, Paisley, UK) selection as described before [21, 22].

Cell proliferation

Cell concentration of B-CPAP and CGTH-w3 in logarithmic growth phase was adjusted to 5×10^4/mL, cultured in a 96-well plate with 200 μL per well. After culture for 24 h, 48 h, 72 h, 96 h. Each group had four repeats. Add to each well 20 μL of fresh medium with 0.5 mg/mL MTT 4 h before termination and continue incubation for 4 h. Add 200 μL Dimethyl Sulfoxide (DMSO) to each well. We took values for each well using 492 nm optical density (OD) [23]. The experiment was repeated for three times.

Wound-healing assay

Cell motility was evaluated using the in vitro wound-healing assay. Cells in exponential growth phase were grown in 24-well plates until they reached confluence. Using a 20 mL plastic pipette tip, we scraped three horizontal lines across the entire diameter at the bottom of each well inducing the 'wound'. Cell media were removed and the cells were gently rinsed three times to remove unattached cells. The wound area was photographed at 24 h after scraping. To compare cell motility of breast cancer cells, we measured the gap distance and determined the wound-closing rate. The cells were allowed to migrate into the wounded area for 24 h. At the indicated time points, the wound closure was photographed by a camera (Model DXM1200, Nikon, Japan) attached to an inverted microscope (Eclipse TE300, Nikon, Japan) [24].

Cell invasion assay

In vitro invasive assays were conducted using the Millicell Cell Culture Insert with 8-μM-pore polyvinylpyrrolidone-free polycarbonate membranes (Millipore, USA) covered with BD MatrigelTM Matrix (15 μg/filter). After growing...
Roles of DJ-1 in papillary thyroid carcinoma cell lines

...to 90% to 100% confluent cultures, B-CPAP and CGTH-w3 cell lines (5×10^5 cells/mL) were trypsinized and resuspended in serum free RPMI-1640 medium containing 1% BSA. From this single-cell suspension, the cells were seeded at 1×10^5 (200 μL cell suspension) in the upper chamber of the Matrigel invasion assay insert. The lower companion plate well contained RPMI 1640 plus 20% FBS. After a 12-hour incubation, the cells on the upper side of the membrane were removed by wiping with a cotton swab. The cells that migrated to the lower side of the membrane were fixed using the methanol and stained with crystal violet for 30 min and then dissolved with 33% acetic acid. The number of cells was indirectly quantitated by measuring the absorbance at 570 nm. Each assay was done in triplicate and repeated at least thrice [24].

Western blot analysis

Protein samples (the same concentration per lane) were separated on a 10-12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and blotted onto polyvinylidene difluoride membranes, blocked in PBST (0.1% triton in 19 PBS) and probed with primary antibodies overnight at 4°C. The membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies. The immunoreactive protein bands were developed by enhanced chemiluminescence. The immunoreactive bands were analyzed by a densitometer [25].

Statistics analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean ± SD. Statistical analyses were performed using SPSS 19.0 statistical software. The P-values were calculated using a one-way analysis of variance (ANOVA). A P-value of <0.05 was considered to indicate a statistically significant result.

Results

DJ-1 high-expressed in PTC cell lines

The western blot and real-time PCR were performed to detect the expression changes of PTC cell lines of B-CPAP, and DGTH-w3. The results summarized in Figure 1A. As we could see, the DJ-1 mRNA levels were higher in B-CPAP, and DGTH-w3 than in control. To test the consequence of the reduced DJ-1 expression on B-CPAP, and DGTH-w3, the DJ-1-specific siRNA (siDJ-1) were transfected. As shown in Figure 1B and 1C, the transfection of siDJ-1 does suppress the DJ-1 mRNA expression.

DJ-1 regulated cells viability of B-CPAP, and DGTH-w3

To further link the expression of DJ-1 with the control of cell proliferation, the cell viability was tested after siDJ-1 transfection. As shown in Figure 2A and 2B, the cell viability significantly decreased after almost 48 h culture. And this confirmed that DJ-1 up-regulated the cell viability.

DJ-1 promotes cell migration

The relative cell migration on B-CPAP and CGTH-w3 was shown in Figure 3. As is shown, the cell migration was decreased after siDJ-1 transfection. The results illustrated the correc-
Roles of DJ-1 in papillary thyroid carcinoma cell lines

**Figure 2.** Down regulation of DJ-1 by siRNA changed the cell viability of thyroid cancer cells. (A) B-CPAP cells were transfected as in (A), cell viability was tested after each 24 h. (B) CGYH-w3 cells were transfected as in (B), cell viability was measured after each 24 h.

**Figure 3.** Correction between cell migration and DJ-1. Migration rate were measured after transfection in B-CPAP and CGYH-w3 respectively.

**Figure 4.** Correction between cell invasion and DJ-1. The number of cells was indirectly quantitated by measuring the absorbance at 570 nm. Each assay was done in triplicate and repeated at least thrice.

**Discussion**

DJ-1 is reported as an oncoprotein that promotes survival of cancer cells through anti-apoptosis, it is a multi-functional protein with anti-oxidant and transcription regulatory activities [26, 27]. So far, many researches and conclusions had demonstrated about the occurrence and development of PTC, including the DJ-1 expression in the papillary thyroid tumor [28, 29]. However, deep research need to be made it clear of the underlying mechanism of PTC. In this study, results have shown that suppression of DJ-1 expression significantly decreased the proliferation, migration and invasion of PTC cells, indicating that DJ-1 may be a key player involved in the progression of PTC. Moreover, the MMP2 and MMP9 expression, as well as P-AKT was significantly inhibited after transfection of siDJ-1.

**Possible pathway**

We further verified the possible pathway of DJ-1 in PTC cells by tested the AKT density after siDJ-1 and overexpression DJ-1 were transfected. As show in Figure 6A and 6B, the P-AKT was significantly inhibited after the knocking down of DJ-1. This suggesting a possibility that DJ-1 influence the cell proliferation, migration and invasion by AKT pathway.

**DJ-1 promotes cell invasion**

Invasive assays in vitro were conducted to demonstrate the correlation of DJ-1 expression with cell invasion which were shown in Figure 4. The results showed that the average of invasive cell number notably decreased by siDJ-1 transfection, this suggesting that the suppression of DJ-1 expression could significantly decrease the invasive ability of B-CPAP and CGYH-w3.

**DJ-1 upregulated MMP2 and MMP9 expression**

The expression of MMP2 and MMP9 was tested using western blot assay. Figure 5A and 5B respectively showed MMP2 and MMP9 relative density, suggesting that the DJ-1 could up regulated these two proteins. Thus, the expression of DJ-1 changed the related protein expression.
Roles of DJ-1 in papillary thyroid carcinoma cell lines

Numerous studies have firmly established that DJ-1 was overexpressed in many kinds of tumors, such as lung carcinoma, ovarian cancer, lung cancer, breast cancer, as well as papillary thyroid carcinoma [30-33]. In the present study, we researched the role of DJ-1 in papillary thyroid carcinoma. To detect the expression changes of PTC cell lines of B-CPAP, and DGTH-w3, the western blot and real-time PCR were performed. The results suggested that DJ-1 was overexpressed in papillary thyroid carcinoma than control. This confirmed the findings before.

We also ask what kind of regulatory mechanism DJ-1 involved in the mediating of PTC. To this end, we researched the connection between DJ-1 and cell viability, cell invasion, and immigration. Our results shown that the reduction of DJ-1 was associated with greater apoptotic cell death, as well as an inhibition of cell migration and invasion ability.

MMPs are a kind of matrix metalloproteinases, promoting tumor invasion and metastasis, regulating host defense mechanisms and normal cell function [34]. The breast cancer cells growth and invasiveness in adjacent and distant sites was proved to be associated with the metalloproteinases (MMPs) expression. Moreover, cell migration and invasion in lung cancer have been proved a connection with the MMPs expression [35]. In order to identify the correction between MMPS and DJ-1 in the PTC, we tested the relative density of MMP2 and MMP9 in B-CPAP and DGTH-w3 after transfection of siDJ-1 and overexpression DJ-1. It turns out that expression of MMP2 and MMP9 were significantly down regulated after the suppression of DJ-1. This made us convince that MMPs may be involved in the process of metastasis of PTC by DJ-1.

DJ-1 was reported may mediate the development and progression of disease through different ways. Ismail IA, et al., reported that DJ-1 induced cell protection and works independent of Nrf2 signaling pathway [36]. Jaramillo-Gómez J, et al., has studied the DJ-1’s involvement in the regulation of the AKT, the results
suggest that DJ-1 reinforces the PI3K/AKT survival pathway and inhibits autophagy, probably by a mechanism independent from mTOR [37]. Since DJ-1 has been shown to be implicated in the regulation of Akt signal, to verify the role of Akt activity in the effect of DJ-1, the Akt and p-akt activity were measured in B-CPAP and DGTH-w3 cells. The results proved that P-AKT has been inhibited after the knocking down of DJ-1, confirming that AKT may partly be associated in the action of DJ-1, it may be the most possible pathway for DJ-1 in PTC.

The results above strongly suggest that AKT may be one of the possible signaling pathways for regulation of proliferation, migration and invasion in the papillary thyroid cancer cells by DJ-1.

In summary, this study stated the mechanism underlying the regulation by DJ-1 thyroid papillary carcinoma. This could be a valuable approach to enhance treatment for thyroid tumor patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bingzong Hou, Department of General Surgery I, Fifth Affiliated Hospital of Sun Yat-sen University, 52 East Meihua Road, Zhuhai 519000, Guangdong, China. Tel: 86-756-2528708; E-mail: houbingzong99663@126.com

References

 Roles of DJ-1 in papillary thyroid carcinoma cell lines


[34] Li X, Wu JF. Recent developments in patent anti-cancer agents targeting the matrix metalloproteinases (MMPs). Recent Pat Anticancer Drug Discov 2010; 5: 109-141.

