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Original Article

The effect of curcumin on the apoptosis of lung cancer cells by regulating DJ-1-PTEN/PI3K/AKT signaling

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Abstract: The PI3K/AKT signaling pathway plays an important role in tumor occurrence. PTEN can negatively regulate the PTEN/AKT signaling pathway, and DJ-1 is the negative regulator of PTEN. DJ-1 upregulation is associated with various tumor occurrences. Curcumin (Cur) is a phenolic compound extracted from various plant rhizomes with an antitumor pharmacological effect. This study investigated the role of Cur in regulating the DJ-1-PTEN/PI3K/AKT pathway activity and lung cancer cell proliferation and apoptosis. Human normal lung epithelial BEAS-2B cells and lung cancer A549 cells were cultured in vitro. Real-time quantitative PCR (qRT-PCR) was used to measure DJ-1 and PTEN mRNA expressions. Western blot was adopted to test DJ-1 and PTEN protein levels. A549 cells were treated with 0, 2.5, 5, and 10 μM of Cur. Cell proliferation activity was assessed using a CCK-8 assay and EdU staining. Cell apoptosis was determined by flow cytometry. A549 cells were divided into a control group, a 10 μM Cur treatment group, and a Cur+PTEN inhibitor SF1670 group. Compared with the BEAS-2B cells, DJ-1 was significantly increased, but the levels of PTEN were clearly decreased in the A549 cells. Cur treatment markedly inhibited A549 cell proliferation and induced apoptosis in a dose dependent manner. Cur downregulated DJ-1 levels and enhanced PTEN expression in A549 cells in a dose dependent manner. The combined administration of SF1670 enhanced the phosphorylation activity of p-AKT, attenuated the inhibitory effect of Cur on the p-AKT protein, and attenuated the pro-apoptotic and proliferative functions of Cur. Cur can inhibit lung cancer cell proliferation and promote apoptosis by downregulating DJ-1 to regulate the activity of PTEN/PI3K/AKT pathway.

Keywords: Curcumin, DJ-1, PTEN/PI3K/AKT, proliferation, apoptosis, lung cancer

Introduction

Lung cancer (LC) is one of the most common human malignancies. It is number one in male and number two in female incidence and mortality [1, 2]. It is a serious threat to human life and brings a huge social burden [3]. The pathogenesis of lung cancer is relatively insidious. Most patients are in an advanced stage when diagnosed, thus missing the best treatment opportunity. Lung cancer is highly malignant, and has rapid disease progression, distant metastasis, a high recurrence rate, and poor survival and prognosis [4, 5].

The phosphatase and tensin homologue deleted on chromosome ten (PTEN) is a negative regulator of the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT/PKB) signaling pathway, so it plays a role in restraining cell proliferation and promoting cell apoptosis [6-9]. The DJ-1/Parkinson gene 7 (PARK7) is a negative regulator of PTEN that can activate the PI3K/AKT signaling pathway by inhibiting PTEN, so it is involved in reducing cell apoptosis, promoting cell proliferation, and regulating survival and progression [10]. Curcumin (Cur) is a polyphenolic substance extracted from the rhizome of *Curcuma longa*. Many studies have found that Cur regulates cell proliferation, apoptosis, migration, and invasion to play an anti-tumor effect [11, 12]. It has been shown that Cur plays a role in regulating the activity of the PTEN-PI3K/AKT pathway [13, 14]. Since DJ-1 is a negative regulator of PTEN, it is unclear whether Cur can regulate the PTEN/PI3K/AKT pathway by targeting DJ-1.

Materials and methods

Main reagents and materials

Human lung cancer A549 and normal lung epithelial BEAS-2B cell lines were purchased from

Shanghai Baili Biotechnology Co., Ltd. RPMI 1640 and penicillin-streptomycin were purchased from HyClone. Fetal bovine serum (FBS) and LHC-8 medium were purchased from Gibco. Trizol was purchased from Invitrogen, and we purchased a PrimeScript™ RT reagent Kit from Takara. Cur was purchased from Sigma. A Cell-Light™ EdU Apollo 488 Flow Cytometry Kit was purchased from Ribobio. Rabbit anti-human DJ-1 and PTEN polyclonal antibodies were purchased from Abcam. Rabbit anti-human β-actin polyclonal antibody was purchased from Santa Cruz. Rabbit anti-human AKT and p-AKT polyclonal antibodies were purchased from CST. HRP-conjugated secondary antibody was purchased from Sangon. A BCA protein quantification kit, a CCK-8 test kit, an Annexin V/PI apoptosis detection kit, and the BeyoECL Plus chemiluminescence reagent were purchased from Beyotime. The PTEN inhibitor SF1670 was purchased from MedChemExpress.

Cell culture

A549 cells were cultured in an RPMI 1640 medium containing 10% FBS and 1% penicillin-streptomycin. BEAS-2B cells were cultured in an LHC-8 medium containing 10% FBS and 1% penicillin-streptomycin. The cells were passaged at 1:3 and used for experiments in the logarithmic phase.

Cell treatment and grouping

The BEAS-2B and A549 cells were cultured in vitro and inoculated into 96-well plates (10000 cells/well). After adhering for 24 hours, they were treated with 0, 2.5, 5, or 10 μM of Cur and cultured for 72 hours. After that, 10 μL of CCK-8 solution was added to each well and the absorbance of each well at 450 nm was measured after 4 hours of reaction (A450). Relative proliferation activity (%) = (A450 value of the treatment group - A450 value of the blank well)/(A450 value of the control group-A450 value of the blank well) × 100%.

Cur and SF1670 treatment

The A549 cells were divided into a control group, a 10 μM Cur treatment group, and a Cur+PTEN inhibitor SF1670 group. The cells were mixed into a 10 μM EdU solution at 37°C for 120 min. After being incubated for 48 h, the cells were digested by trypsin and collected. After being fixed in 4% paraformaldehyde for 15-30 min and neutralized in 2 mg/mL glycine for 5 min, the cells were stained in 500 μL Apollo solution and resuspended in PBS. Finally, the cells were centrifuged at 300 g for 5 min, and then the cells were tested on a Beckman FC500 MCL flow cytometer.

Cell apoptosis detection

The cells were resuspended in 100 μl of binding buffer and incubated in 5 μl of annexin V-FITC and 5 μl PI in the dark for 15 min. Next, the cells were mixed with 5 μl PI and tested using flow cytometry to evaluate cell apoptosis.

qRT-PCR

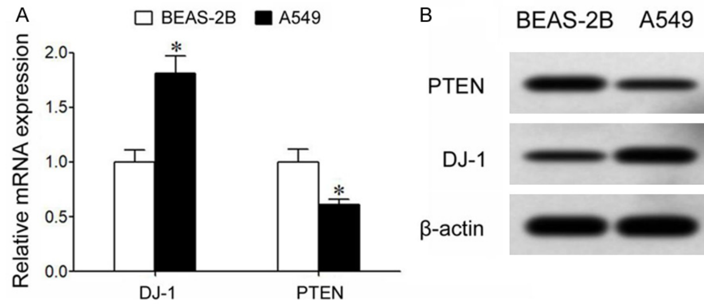
Total RNA was extracted using Trizol and reverse transcribed using a PrimeScript™ RT reagent Kit. The reaction system contained 5.0 μL 2×SYBR Green Mixture, 0.5 μL primer at 5 μm/L, 1 μL cDNA, and ddH_2O. The reverse transcription was performed at 50°C for 15 min and 85°C for 5 min. The PCR reaction was composed of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time PCR was performed on Bio-Rad CFX96 to test the relative expression.

Western blot

Total protein was extracted by RIPA from cells. A total of 40 μg protein was separated by 10% SDS-PAGE for 3 h and transferred to a PVDF membrane at 300 mA for 90 min. Next, the membrane was blocked and incubated in a primary antibody at 4°C overnight (DJ-1, PTEN, AKT, p-AKT, and β-actin at 1:1000, 1:800, 1:1000, 1:500, and 1:10000, respectively). Then the membrane was incubated in a secondary antibody (1:10000) for 60 min after being washed with PBST for three times. Finally, the protein expression was measured using ECL chemiluminescence.

Statistical analysis

All data analyses were performed using SPSS 18.0 software. The measurement data were depicted as the mean ± standard deviation and



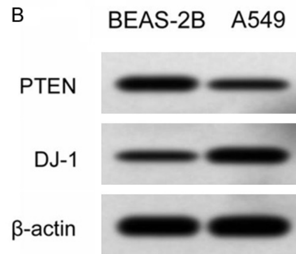


Figure 1. DJ-1 is upregulated, but PTEN is decreased in lung cancer cells. (A) qRT-PCR quantification of mRNA expression; (B) Western blot detection of protein expression. $^{*}P < 0.05$, compared with BEAS-2B cells.

compared using a *t* test or a one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

DJ-1 upregulated, but PTEN decreased in lung cancer cells

A qRT-PCR showed that, compared with normal lung epithelial BEAS-2B cells, DJ-1 mRNA was significantly increased, but PTEN mRNA was clearly decreased in the A549 cells (**Figure 1A**). Western blot demonstrated that the DJ-1 protein was markedly enhanced, but the PTEN protein was decreased in the A549 cells compared with normal lung epithelial BEAS-2B cells (**Figure 1B**).

Cur treatment significantly inhibited lung cancer cell proliferation

A CCK-8 assay revealed that different concentrations of Cur showed a similar impact on the proliferative activity of normal lung epithelial BEAS-2B cells (**Table 1**). Cur treatment markedly inhibited A549 cell proliferation and induced apoptosis in a dose dependent manner (**Table 1**) (**Figure 2**).

Cur treatment suppressed DJ-1 expression and upregulated PTEN expression in A549 cells

A qRT-PCR showed that Cur downregulated the DJ-1 mRNA level and enhanced the PTEN mRNA expression in the A549 cells in a dose dependent manner (**Figure 3A**). Western blot found that Cur reduced the DJ-1 protein level and upregulated PTEN protein expression in A549 cells (**Figure 3B**).

Cur inhibited lung cancer cell proliferation and induced cell apoptosis, but SF1670 can antagonize the effect of Cur

A qRT-PCR showed that Cur treatment significantly downregulated DJ-1 mRNA and elevated PTEN mRNA in A549 cells. The combination of SF1670 treatment did not affect DJ-1 and PTEN mRNA expressions in A549 cells (**Figure 4A**). Western blot showed that the combination of SF1670 treatment did not affect the regulation of Cur on DJ-1 and PTEN protein expression, but it significantly enhanced the phosphorylation activity of the downstream AKT protein (**Figure 4B**). Flow cytometry showed that Cur significantly inhibited A549 cell proliferation (**Figure 4D**) and increased cell apoptosis (**Figure 4C**). SF1670 combined with Cur attenuated the inhibition of Cur on A549 cell proliferation and significantly reduced apoptosis.

Discussion

When the PI3K/AKT pathway is activated, P13K can be transformed to phosphatidylinositol 3,4,5-triphosphate (PIP3) by catalyzing phosphatidylinositol 4,5-trisphosphate (PIP2). PIP3 recruits AKT from the cytoplasms to the membranes, and it phosphorylates loci Ser473 and Thr308 of AKT under the action of phosphoinositide-dependent protein kinase (PDK). Phosphorylation-activated AKT further activates downstream signaling molecules to participate in cell growth, survival, and apoptosis by regulating the transcription and expression of various downstream genes [15]. It was shown that the excessive activation of the PI3K/AKT pathway is associated with the development, progression, metastasis, and drug resistance of various tumors, such as endometrial cancer [16], gastric cancer [17], and lung cancer [18]. The most important substrate for PTEN is PIP3, which maintains low levels by dephosphorylating PIP3 and antagonizing PI3-K's impact on PIP2 phosphorylation, thereby inhibiting PI3K activation via PIP3 phosphorylation. DJ-1 is a negative regulator of PTEN, which attenuates the inhibitory effect of PTEN on the PI3K/AKT signaling pathway by suppressing the expression and function of PTEN, thereby indirectly activating the PI3K/AKT signaling path-

Table 1. The impact of Cur on cell proliferation

		0 μM	2.5 μM	5 μM	10 μM
Relative proliferative rate (%)	BEAS-2B	100 ± 6.89	96.63 ± 7.45	106.39 ± 10.13	98.81 ± 8.77
	A549	100 ± 7.56	91.32 ± 8.76	78.29 ± 8.52	59.92 ± 6.31
Apoptotic rate (%)	BEAS-2B	1.63 ± 0.12	2.06 ± 0.19	1.95 ± 0.20	2.39 ± 0.25
	A549	2.07 ± 0.18	8.36 ± 1.13	13.23 ± 1.45	22.58 ± 3.06

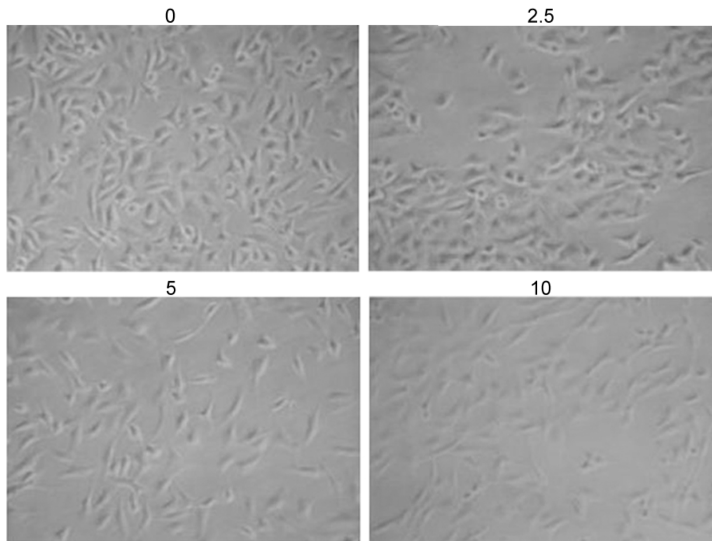


Figure 2. Cell morphology after treatment with different concentrations of Cur (0, 2.5, 5, and 10 mM) (x 40). A representative image is shown from three independent experiments.

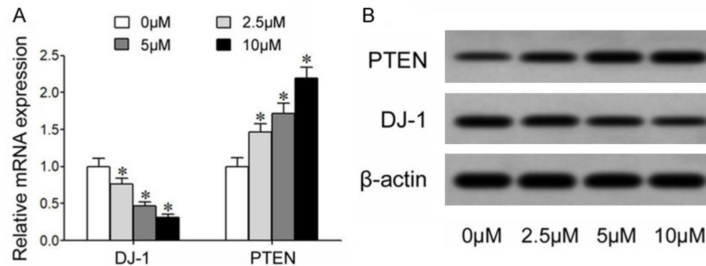


Figure 3. Cur treatment suppressed DJ-1 expression and upregulated PTEN expression in A549 cells. (A) qRT-PCR quantification of mRNA expression; (B) Western blot detection of protein expression. *P < 0.05, compared with 0 μM group.

way, reducing apoptosis, and promoting cell proliferation [10].

Increasing evidence has demonstrated that Cur has anti-tumor effects, such as inhibiting tumor cell proliferation, promoting tumor cell apoptosis, anti-angiogenesis, and restraining invasion and migration [11, 12]. It was shown that Cur plays a role in regulating the PTEN-PI3K/AKT pathway [13, 14]. Since DJ-1 is a negative regulator of PTEN, it is unclear whether Cur may affect the PTEN/PI3K/AKT pathway by regulating DJ-1. This study investigated the role of Cur in regulating the DJ-1-PTEN/PI3K/AKT pathway activity and lung cancer cell proliferation and apoptosis.

Our results showed that, compared with the normal lung epithelial cell BEAS-2B, DJ-1 mRNA was significantly increased, but PTEN mRNA was clearly declined in the A549 cells, revealing that DJ-1 downregulation plays a role in enhancing PTEN expression and the regulation of lung cancer. Han *et al.* [19] showed that the serum levels of DJ-1 were significantly elevated in lung cancer patients compared with healthy controls and benign lung disease. Vavougios *et al.* [20] reported that the content of the DJ-1 protein in the pleural effusion of lung cancer patients was significantly increased with age dependence. Zeng *et al.* [21] used a proteomic analysis to show that the expression of DJ-1 in DDP-resistant lung cancer A549/DDP cells was clearly higher than the expression of parental lung cancer A549 cells. A clinical investigation showed that the increased expression of DJ-1 in tumor tissues was associated with cisplatin chemoresistance in patients. The higher the DJ-1 expression was, the worse the patient's prognosis was. Bai *et al.* [22] found that the expression level of DJ-1 in high-metastasis lung cancer Anip973 cells was markedly higher than the expression of low-metastasis lung cancer AGZY83-a cells. Compared with normal lung tissue, the expression of DJ-1 was apparently increased in lung cancer tissues. Increased DJ-1 expression was

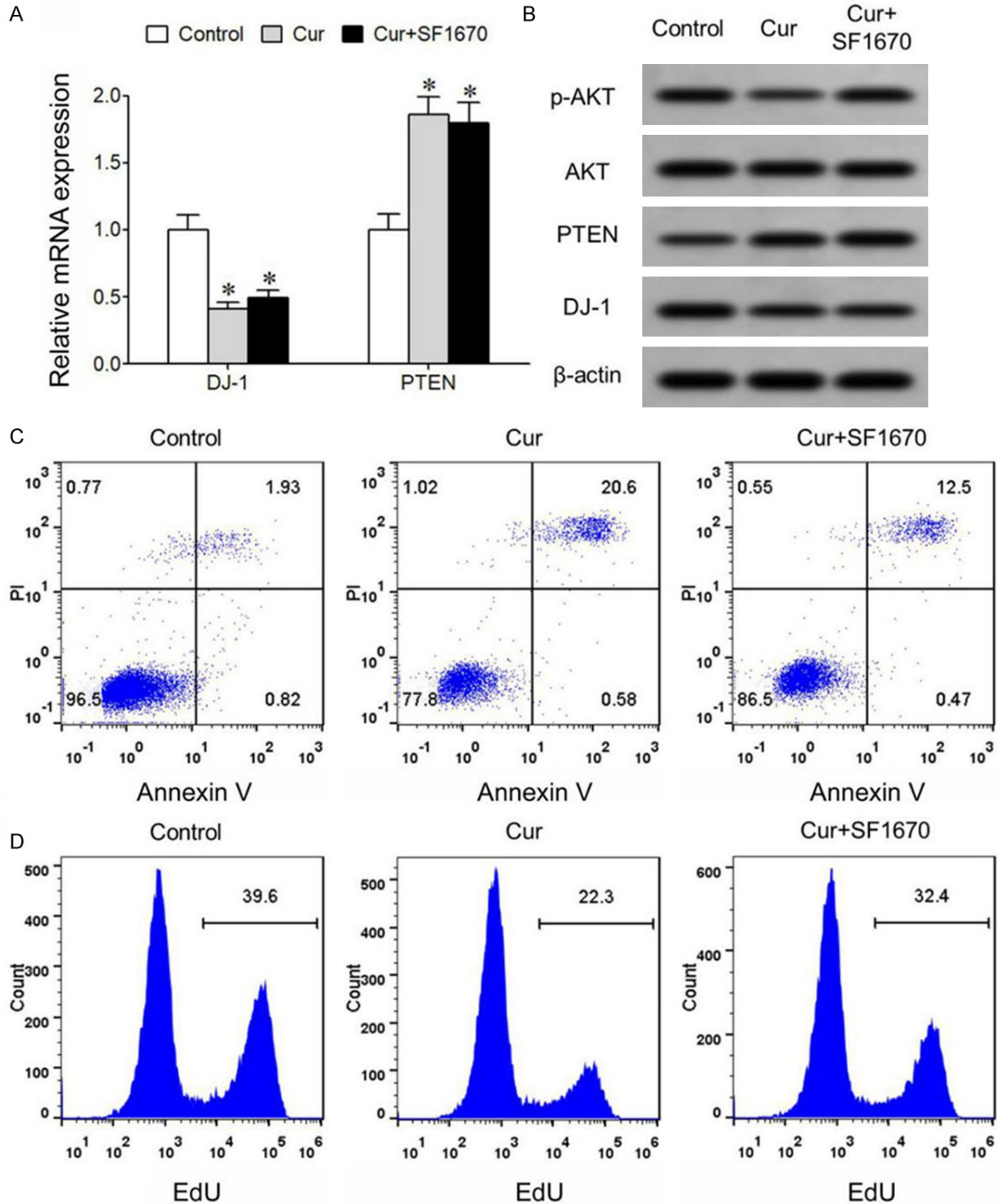


Figure 4. Cur inhibited lung cancer cell proliferation and induced cell apoptosis, but SF1670 can antagonize the effect of Cur. (A) qRT-PCR measurement of mRNA expression; (B) Western blot detection of protein expression; (C) Flow cytometry detection of cell apoptosis; (D) EdU staining detection of cell proliferation. *P < 0.05, compared with the control.

associated with lymph node metastasis in lung cancer. Gao *et al.* [23] revealed that the expression of DJ-1 in the tumor tissues of lung cancer patients was abnormally increased, which was related to their poor prognosis (χ^2=7.946, P=0.005). The above studies indicated that an elevated expression of DJ-1 plays an oncogenic role in the pathogenesis of lung cancer, which is similar to our findings.

In this study, different concentrations of Cur significantly inhibited A549 cell proliferation,

promoted cell apoptosis, downregulated DJ-1 expression, and elevated PTEN levels. It indicated that Cur exerts an anti-tumor effect on lung cancer by restraining DJ-1 expression to enhance PTEN levels. We further combined the application of the PTEN inhibitor SF1670 on the basis of Cur. It was observed that the combination of SF1670 treatment enhanced the expression of the p-AKT protein and attenuated the effect of Cur on cell proliferation inhibition and apoptosis induction, revealing that the downregulation of DJ-1 and the upregulation of PTEN by Cur plays a role in inhibiting the activity of the PI3K/AKT pathway and attenuating the malignant biological characteristics of lung cancer cells. Zhang *et al.* [24] showed that treatment with Cur significantly increased the expression of PTEN, inhibited tumor cell proliferation, and induced apoptosis, but the treatment was significantly attenuated by PTEN siRNA. Yang *et al.* [25] found that a Cur analog can suppress prostate cancer DU145 cell proliferation and induce apoptosis by significantly upregulating the expression of PTEN by targeting miR-21. Gawde *et al.* [26] showed that a Cur analog exerted the anti-tumor effect of inhibiting cell proliferation and promoting apoptosis by significantly enhancing PTEN expression. They revealed that Cur plays an important role in regulating PTEN and in affecting the biological effects of tumor cells, but whether Cur regulates DJ-1 has not been reported. Zeng *et al.* [21] demonstrated that siRNA interfered with DJ-1 expression in cisplatin (DDP)-resistant lung cancer A549/DDP cells, clearly elevating the drug sensitivity and enhancing the proliferation inhibition effect of DDP. Gao *et al*. [23] reported that DJ-1 siRNA significantly reduced drug resistance, increased cell apoptosis, and arrested the cell cycle in the G0/G1 phase of lung cancer H69AR cells. This study observed that Cur can affect the activity of the PTEN/PI3K/AKT pathway by downregulating DJ-1 to exert an anti-tumor effect on lung cancer.

Conclusion

Cur can inhibit lung cancer cell proliferation and promote apoptosis by downregulating DJ-1 to regulate the activity of the PTEN/PI3K/AKT pathway.

Disclosure of conflict of interest

None.

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