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ORIGINAL ARTICLE

Curcumin inhibits proliferation and invasion of papillary thyroid carcinoma cells by inhibiting the JAK2 / STAT3 pathway

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Summary

Purpose: *The purpose of this study was to analyze the function of curcumin to suppress the proliferative and invasive abilities of papillary thyroid carcinoma (PTC) through inhibiting the JAK2/STAT3 pathway.*

Methods: *After treatment of different doses of curcumin in TPC-1 and SW1736 cells, changes in viability, clonality, cell cycle, apoptosis, wound healing and invasion were determined. Western blot analyses were performed to detect protein levels of apoptosis-associated genes, JAK2 and STAT3 in TPC-1 and SW1736 cells treated with different doses of curcumin.*

Results: *Curcumin treatment dose-dependently reduced viability, clonality and metastatic ability in TPC-1 and SW1736 cells. After treatment of 10 μM or 20 μM curcumin, PTC cells were blocked in G2/M phase, and their apoptotic rate increased. Curcumin treatment downregulated Bcl-2 and upregulated Bax in PTC cells. In addition, curcumin treatment downregulated p-JAK2 and p-STAT3 in TPC-1 and SW1736 cells.*

Conclusions: *Curcumin treatment blocks PTC cells to proliferate and invade via inhibiting the JAK2/STAT3 pathway.*

Key words: *curcumin, PTC, proliferation, invasion*

Introduction

As the most common malignancy of the endocrine system, thyroid carcinoma accounts for about 1% of the total number of malignant tumors [1]. Papillary thyroid carcinoma (PTC) is the leading prevalent subtype, accounting for approximately 80% of all thyroid cancer cases [2]. Generally speaking, patients with early-stage PTC have a very good prognosis after surgery. The 5-year survival of PTC is up to 97%. Nevertheless, 10-15% of PTC patients have a poor prognosis due to distant metastasis and recurrence [3]. Currently, clinical treatments for PTC include surgical resection, radioactive I-131 therapy [4], chemotherapy and combination therapy [5]. Most of therapeutic strategies for PTC show great efficacy. However, a small part of PTC patients, especially those carrying BRAFV600E mutation, suffer of poor outcome and high rate of recurrence [6]. Therefore, it is necessary to develop effective drugs targeting PTC. Natural medicines are characterized with extensive efficacy, low toxicity and low cost, and have been well concerned as promising anti-tumor drugs.

Main phytochemicals in the diet have been highlighted due to their obvious safety and effectiveness [7]. Curcumin is a polyphenolic compound extracted from the roots of Curcuma Longa, which has been applied in the traditional Chinese Medicine for a long period [8]. The anti-proliferation,

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anti-oxidant, anti-inflammatory and anti-tumor properties of curcumin have been identified [9]. In many types of tumors, curcumin exerts extraordinary anti-tumor activities through regulating tumor cell behaviors [10-12]. Importantly, clinical trials have proved its low toxicity and high safety. Uncontrolled proliferation and apoptosis would accelerate the malignant progression of tumors [13,14]. A previous study demonstrated the inhibitory effect of curcumin on PTC progression through blocking its proliferative ability and inducing apoptosis [15]. In K1 PTC, curcumin administration suppresses invasion and metastasis of tumor cells [16,17]. Curcumin is able to inhibit hypoxia-induced migratory acceleration in PTC as well [18].

In this paper, we mainly explored the potential function of curcumin in affecting many phenotypes of PTC cells and the underlying mechanism.

Methods

Cell culture

PTC cells TPC-1 and SW1736 were purchased from Cell Bank (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Curcumin (99% purity, Sigma-Aldrich Co., St. Louis, MO, USA) was diluted in dimethyl sulfoxide (DMSO), and preserved at -20°C in the dark. PTC cells were treated with 5, 10 or 20 μM curcumin for 24 h, respectively.

Cell counting kit-8 (CCK-8)

Cells were inoculated in 96-well plates with 2×10^3 cells/well. At the appointed time points, absorbance value at 450 nm of each sample was recorded using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for plotting the viability curves.

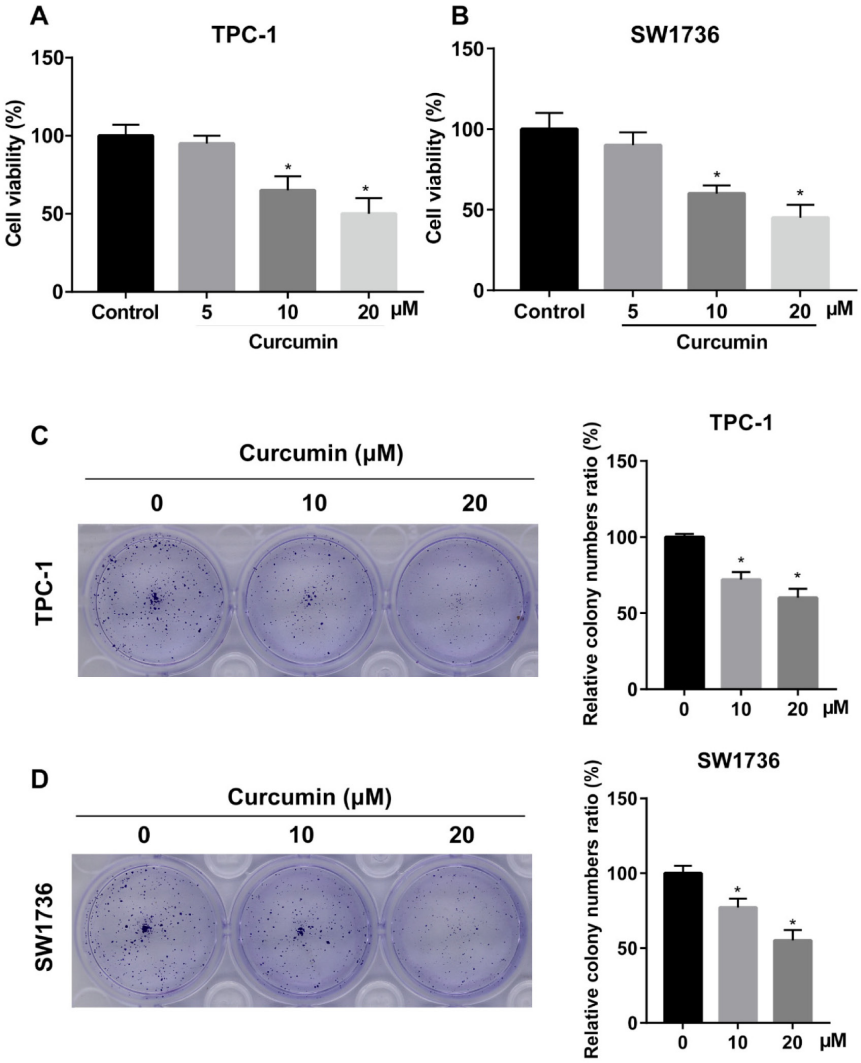

Figure 1. Curcumin treatment dose-dependently inhibited proliferative ability in PTC. **A:** Viability in TPC-1 and SW1736 cells and **B:** after 0, 5, 10 or 20 μM curcumin treatment for 24 h. **C:** Relative colony number in TPC-1 and SW1736 cells and **D:** after 0, 10 or 20 μM curcumin treatment for 24 h.

Colony formation assay

1×10^3 cells/well were inoculated in 6-well plates, induced with different doses of curcumin for 24 h and cultured for 10 days. Afterwards, cells were fixed with 4% polyformaldehyde for 30 min and dyed with 0.1% crystal violet for 30 min. Visible colonies were finally captured.

Cell cycle determination

Cells were washed with phosphate buffered saline (PBS) twice, digested and prepared to cell suspension with 1×10^5/mL. After fixation in 70% ethanol, cells were dyed with 500 μL of propidium iodide (PI) at 4°C, in the dark for 15 min. Finally, cell cycle distribution was analyzed.

Flow cytometry

Cells were washed with PBS twice, digested and prepared to cell suspension with 1×10^5/mL. Cells were incubated in 1 ml of pre-cold 70% ethanol at 4°C overnight. In the next day, cells were incubated with 100 μL of RNaseA at 37°C, in the dark for 30-min water bath. Subsequently, cells were dyed with 500 μL of PI at 4°C in the dark for 15 min and finally the apoptotic rate was determined at 488 nm wavelength.

Western blot

Cellular protein was isolated using radioimmunoprecipitation assay (RIPA) and electrophoresed. Protein sample was loaded on polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were immersed in PBS containing 5% skim milk for 2 h, reacted with primary antibodies at 4°C overnight and secondary antibodies for 2 h. Band exposure was achieved by electrochemiluminescence (ECL) and processed by Image Software (NIH, Bethesda, MD, USA).

Wound healing assay

Cells were seeded in 6-well plates with 1.0×10^6 cells/well. An artificial wound was created in the confluent cell monolayer using a 100 μL pipette tip. Wound closure was captured at 0 and 24 h for calculating the percentage of wound width.

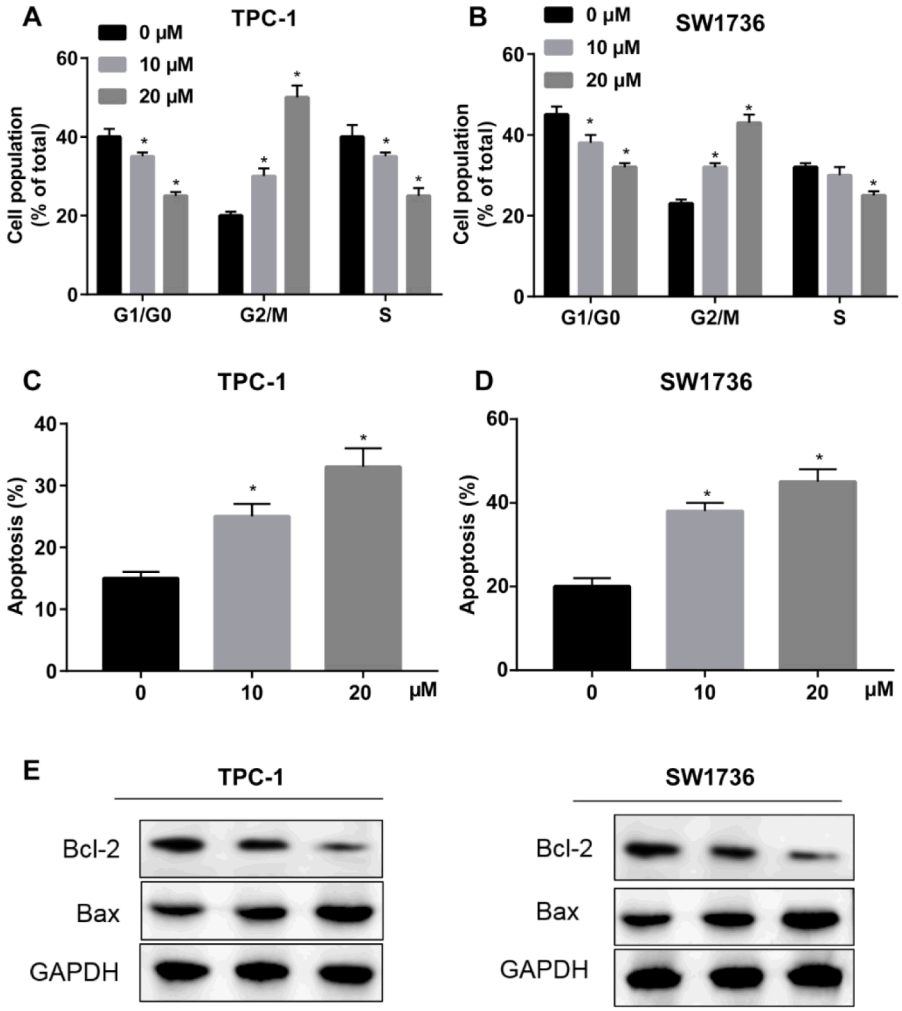

Figure 2. Curcumin treatment induced apoptosis in PTC. **A,B:** Cell cycle distribution in TPC-1 **(A)** and SW1736 cells **(B)** after 0, 10 or 20 μM curcumin treatment for 24 h. **C,D:** Apoptotic rate in TPC-1 **(C)** and SW1736 cells **(D)** after 0, 10 or 20 μM curcumin treatment for 24 h. **E:** Protein levels of Bcl-2 and Bax in TPC-1 and SW1736 cells after 0, 10 or 20 μM curcumin treatment for 24 h.

Transwell assay

3×10^4 cells were inoculated on the top of a transwell insert placed in 24-well plates while 100 μL of serum-free medium was applied in the bottom. After 24-h incubation, cells invaded to the bottom side were immersed in methanol for 15 min, dyed with crystal violet for 20 min and counted using a microscope. Invasive cell number was counted in 5 randomly selected fields per sample (magnification 40×).

Statistics

SPSS 22.0 (IBM, Armonk, NY, USA) was used for statistical analyses. Data were expressed as mean ± SD (standard deviation). Comparison between multiple groups was done using one-way ANOVA test followed by *post hoc* test (least significant difference). $P<0.05$ indicated significant difference.

Results

Curcumin treatment dose-dependently inhibited proliferative ability in PTC

To uncover potential influences of curcumin on the proliferation of PTC, TPC-1 and SW1736 cells were treated with 0, 5, 10 or 20 μM curcumin for 24 h, respectively. CCK-8 results depicted a dose-dependent decline in cell viability (Figures 1A,1B). Meanwhile, relative colony numbers were reduced after 0, 10 or 20 μM curcumin treatment for 24 h (Figures 1C,1D). Therefore, curcumin treatment dose-dependently attenuated the proliferative ability in PTC.

Curcumin treatment induced apoptosis in PTC

TPC-1 and SW1736 cells were treated with 0, 10 or 20 μM curcumin for 24 h. Flow cytometry revealed that cells were remarkably arrested in G2/M phase after curcumin treatment (Figures 2A,2B). The apoptotic rate was dose-dependently elevated in PTC cells treated with 10 or 20 μM curcumin for 24 h (Figures 2C,2D). In addition, western blot analyses showed that Bcl-2 was gradually downregulated, while Bax was dose-dependently upregulated in PTC cells following curcumin treatment (Figure 2E). It is suggested that the apoptotic ability of PTC was stimulated by curcumin treatment.

Curcumin treatment dose-dependently inhibited migratory and invasive abilities in PTC

Metastatic ability changes in PTC influenced by curcumin treatment were assessed by wound healing assay and transwell assay. The percentage of wound width gradually increased in PTC cells treated with 0, 10, and 20 μM curcumin, respectively, revealing the suppressed migratory ability (Figures 3A,3B). Transwell assay revealed a dose-dependent decline in invasive cell number after curcumin treatment (Figures 3C,3D).

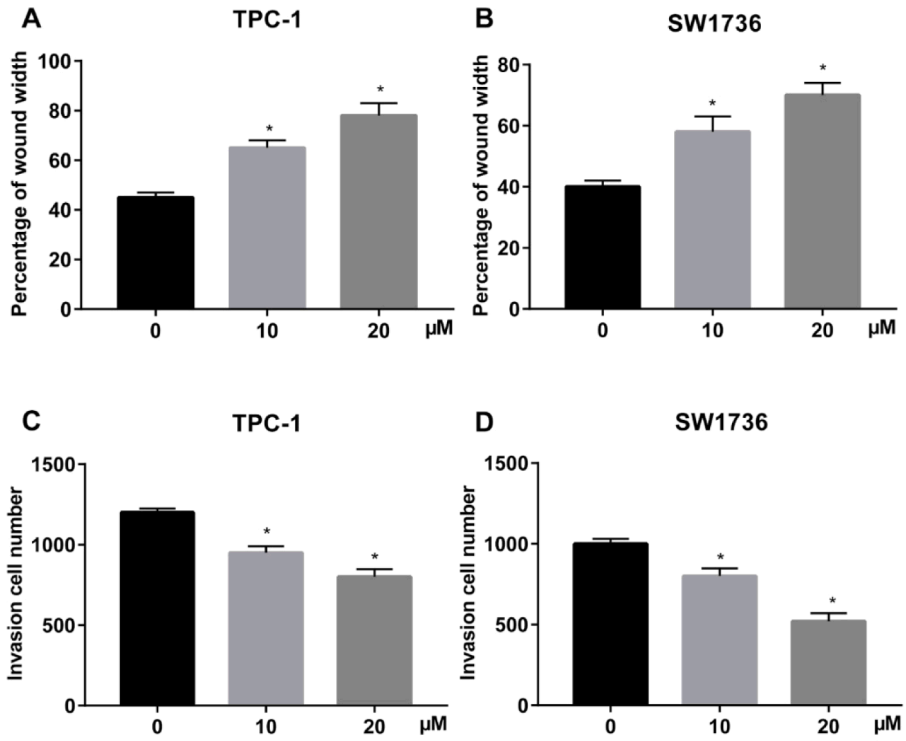

Figure 3. Curcumin treatment dose-dependently inhibited migratory and invasive abilities in PTC. **A,B:** Percentage of wound width in TPC-1 **(A)** and SW1736 cells **(B)** after 0, 10 or 20 μM curcumin treatment for 24 h. **C,D:** Invasive cell number in TPC-1 **(C)** and SW1736 cells **(D)** after 0, 10 or 20 μM curcumin treatment for 24 h.

Curcumin treatment inactivated the JAK2/STAT3 pathway

The JAK/STAT3 pathway is critical during the malignant progression of PTC [19]. Herein, we determined protein levels of p-JAK2, JAK2, p-STAT3 and STAT3 in curcumin-treated TPC-1 and SW1736 cells. Curcumin treatment dose-dependently reduced the ratios of p-JAK2/t-JAK2 and p-STAT3/t-STAT3 in TPC-1 and SW1736 cells, showing suppressed JAK2/STAT3 pathway (Figures 4A,4B).

Discussion

The incidence of thyroid carcinoma has increased significantly in the past decade. PTC is the major subtype of thyroid carcinoma [20]. Surgical resection and radioactive I-131 therapy are the two effective strategies for PTC. However, tumor metastasis and recurrence increase the risk of surgical procedures and limit the prognosis of PTC [21]. Drugs that could effectively inhibit PTC metastasis are urgently required.

Curcumin is an active ingredient of Curcuma Longa with a variety of pharmacological activities [22]. Curcumin is capable of suppressing invasive and migratory abilities of many types of tumor cells [23]. Tumor metastasis is a complex, multistep process. Initially, tumor cells invade, migrate and infiltrate other regions, and thus the distant colonization triggers tumor angiogenesis and the formation of distant metastases [24]. In this study, curcumin treatment dose-dependently reduced viability, arrested cell cycle in G2/M phase and induced apoptosis in PTC. Meanwhile, curcumin treatment attenuated migratory and invasive abilities in PTC. We believed that curcumin treatment could effectively prevent metastasis of PTC.

The JAK/STAT pathway was initially discovered in the interferon pathway [25,26]. JAK2 is a tyrosine kinase involved in the JAK/STAT pathway and has been demonstrated to be able to induce tumor cell survival and angiogenesis [27,28]. STAT3 is closely related to tumor progression through affecting malignant phenotypes of tumor cells [29-

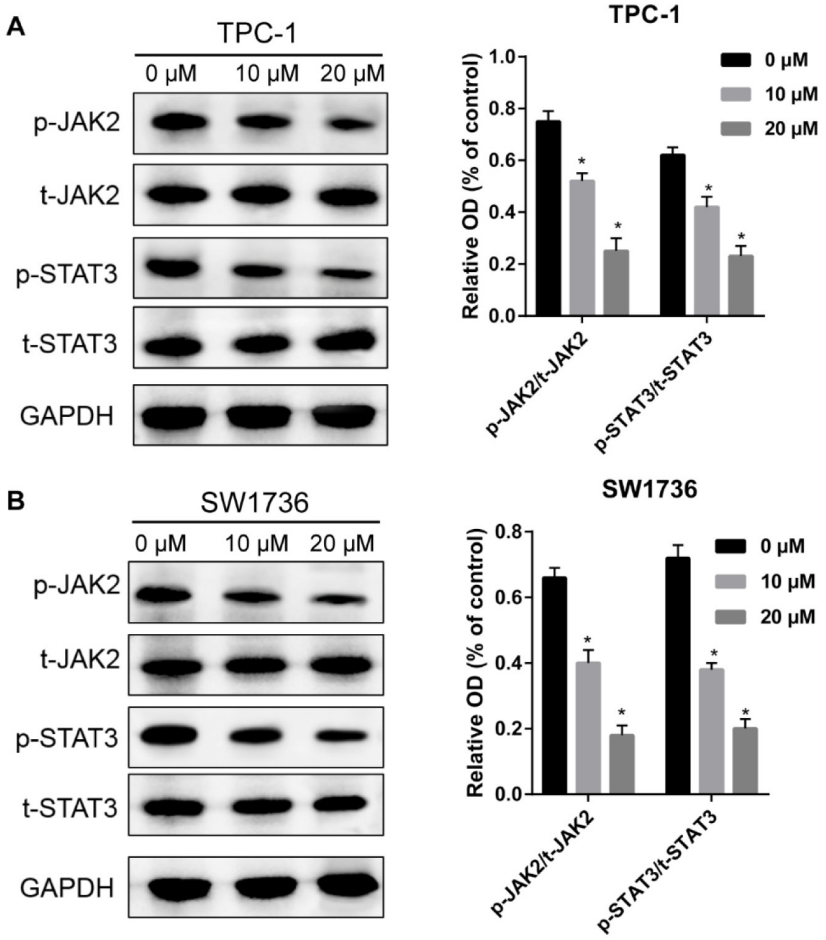

Figure 4. Curcumin treatment inactivated the JAK2/STAT3 pathway. Protein levels of p-JAK2, JAK2, p-STAT3 and STAT3 in TPC-1 after 0, 10 or 20 μM curcumin treatment for 24 h. Curcumin markedly reduced the ratios of p-JAK2/t-JAK2 and p-STAT3/t-STAT3 in TPC-1 **(A)** and SW1736 **(B)** cells; *p<0.05.

31]. The abnormal activation of JAK/STAT pathway is proved to be related to many types of tumors [19,32-35]. Our findings illustrated that curcumin treatment reduced the ratios of p-JAK2/t-JAK2 and p-STAT3/t-STAT3 in TPC-1 and SW1736 cells, indicating the suppressed JAK-STAT pathway. Based on our results, it is suggested that curcumin treatment attenuated viability and metastasis in PTC through inactivating the JAK/STAT pathway.

Conclusions

Curcumin treatment blocks PTC cells to proliferate and invade *via* inhibiting the JAK2/STAT3 pathway.

Conflict of interests

The authors declare no conflict of interests.

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