Effects of Curcumin on Invasion and Metastasis in the Human Cervical Cancer Cells Caski

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CLC number: R730.23 Document code: A Article ID: 1000-9604(2009)02-0159-04

DOI: 10.1007/s11670-009-0159-8

ABSTRACT

Objective: To explore the effects of curcumin on invasion and metastasis in the human cervical cancer cells Caski.

Methods: Caski cells were treated with 10, 25, 50μmol/L curcumin for 24, 48, 72 h. Proliferation of Caski cells was measured with MTT assay. When treated with 50μmol/L curcumin for 72 h, the expressions of MMP-2, MT1-MMP and NF-κB of cells were detected by Western-blot, and invasion and metastasis of Caski cells were evaluated with transwell chamber.

Results: After being treated with 10μmol/L, 25μmol/L, 50μmol/L curcumin for 24, 48 and 72 h, the proliferation of Caski cells was inhibited in a dose-and time-dependent manner. The expression of MMP-2, MT1-MMP and NF-κB were decreased when being treated with 50μmol/L curcumin for 72 h. After treatment with 50μmol/L curcumin, in invasion assay, the number of cells in curcumin treated group to migrate to filter coated with Matrigel was reduced compared with control group(P<0.05). Meanwhile, in migration assay, the number of cells in curcumin treated group to migrate to filter was also decreased compared with control group (P<0.05).

Conclusion: Curcumin could affect the invasion and metastasis of the human cervical cancer cells Caski. Inhibiting the expression of MMP-2, MT1-MMP and NF-κB was probably one of its molecular mechanisms.

Key words: Cervical cancer; Curcumin; Invasion; Metastasis

INTRODUCTION

Curcumin, a deferuloymethane, is a major active component of the food flavor turmetric(Curcuma Longa).Because of its stable colour and luster and low toxicity, curcumin has been widely used as food additive and stain. Several studies demonstrated that curcumin has anticarcinogenic[1], antioxidant[2], anti-inflammation[3] and antiangiogenic properties[4], and can modulate mutidrug- resistance gene and protein functions[5]. In recent years, it has also been reported that curcumin reduced cancer cells invasion *in vitro* and *in vivo*[6,7]. But the mechanisms remain unclear. The regulation of matrix metalloproteinases (MMPs) play an important role in cancer cells invasion by cleavage of extracellular matrix (ECM). In this study, we examined the effects of curcumin on the expressions of MMP-2, MT1-MMP and NF-κB, and the invasion and metastasis of human cervical cancer cells Caski.

Received: Oct. 15, 2008; **Accepted:** Feb. 24, 2009
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MATERIALS AND METHODS

Materials

Caski cell line was supplied by department of pathophysiology of Chong qing medical University. Curcumin and 3-(4,5-demethy-2-thiazolyl)-2,5-dephenyl-2h-tetrazolium-bromid (MTT) were obtained from Sigma (St Louis, Mo, USA). RPMI-

1640 and fetal bovine serum(FBS) were from Hyclone. The mouse monoclonal anti-MMP-2, anti-MT1-MMP and anti-NF-κB antibodies were purchased from Santa Cruz (USA).

Cells Culture and Experiment Groups

Caski cells were maintained in RPMI-1640 containing 10%FBS and antibiotic-antimycotic. The cells were cultured in a humidified incubator in 5% CO_2, 37°C in air. For all the experiments, the cells were divided into control group (not treated with curcumin) and treated groups (treated with 10, 25, 50 μmol/L curcumin, respectively).

MTT Assay

Cell growth was measured by a modified MTT assay. About 1×10^5 cells /well were plated in 96-well microplates and incubated overnight. Cells were then treated with 10, 25 and 50μmol/L curcumin for 24, 48 and 72 h. Then 20 μl stock MTT was added to each well and the cells were further incubated at 37°C for 4 h. The supernatant was removed and 200μl DMSO in isopropanol was added to each well to solubilize the formazan products. The absorbance at wavelength of 570nm was measured by a micro ELISA reader (Sigma). The negative control well contained medium only. The ratios of the absorbance of treated cells relative to those of the control wells were calculated and expressed as percentage of growth inhibition.

***In vitro* Invasion Assay**

Caski cells were treated with 50μmol/L curcumin for 72 h. *In vitro* invasion assay was performed using 24-well transwell unit with polycarbonate filters (Corning Costar, Cambridge, MA). The upper side of filter was coated with Matrigel (BD, USA). Lower compartment was filled with supernant of NIH3T3. Cells were placed in the upper part of the transwell unit, incubated for 48 h, fixed with 95% alcohol and stained with hematoxylin for 10 min followed briefly by eosin. The invasive phenotypes were determined by counting the cells that migrated to the lower side of the filter with microscopy at ×400. Five fields were counted for each filter and each sample was assayed in triplicate.

***In vitro* Migration Assay Using Transwell**

In vitro migration assay was also performed using a 24-well transwell unit with polycarbonate filters. Experimental procedures were the same as the in vitro invasion assay described above except that the filter was not coated with Matrigel for the migration assay.

Western Blot

Caski cells were treated with 50μmol/L curcumin for 72h. After culture solution was discarded, about 5×10^6 cells of control group and treated group were collected. The solution (containing 0.1mol/L NaCl, 0.01 mol/L Tris HCl, ph7.6, 0.001 mol/L EDTA, 100g/ml PMSF and 2μg/ml Leupeptin) was used to lyse the cells for 30min. After centrifugation at 10000r/min for 10min, the supernatant was collected. All above-mentioned operations were done under 4°C. Protein concentration was measured and samples with 25μg protein were loaded and run on SDS polyacrylamide. Proteins were then transferred onto nitrocellulose membrane. The membrane was blocked with 5% fat-free milk for 1h, then probed with antibodies against MMP-2, MT1-MMP, NF-κB (1:300) and β-actin (1:1000) and kept at 4°C overnight. After being washed three times with TBS for 10 min, the membrane was incubated with horseradish peroxidase (HRP) conjugated goat anti-mice IgG(1:1000) for 2h at 37°C. After washing immunoreactive bands were visualized by ECL reagent. The results were analyzed by Quantity One software (Bio-Rad, USA). The experiments were performed more than three times.

Statistical Analysis

All data are expressed as $\bar{x}\pm s$. Statistical differences between the various groups were assessed with a one-way ANOVA followed by a post hoc test. Comparisons between two groups were assessed by unpaired *t* test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Effects of Curcumin on the Proliferation of Caski Cells by MTT

Caski cells treated with different concentrations of curcumin for 24, 48 and 72 h resulted in the inhibition of cell proliferation in a dose-and time-dependent manner. The results showed great differences between the control group and the curcumin treated groups ($P<0.05$, Figure 1).

Effects of Curcumin on Invasion and Migration of Caski Cells

As can be seen in Table 1, after being treated

with 50μmol/L curcumin, the invasion and migration of Caski cells were significantly decreased. In invasion assay, the number of cells in curcumin treated group to migrate to filter coated with Matrigel was reduced compared with control group (P<0.05). Meanwhile, in migration assay, the number of cells in curcumin treated group to migrate to filter was also decreased compared with control group (P<0.05).

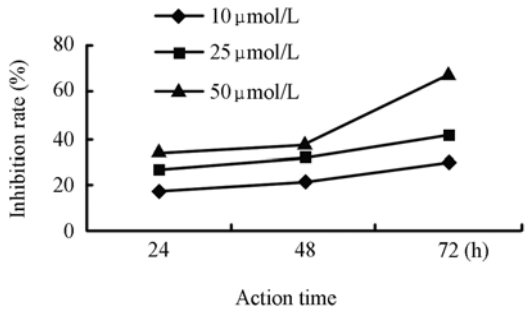

Figure 1. Inhibition of curcumin on Caski cells growth.

Table 1. Effects of curcumin on invasion and migration of Caski cells
(%, n=9, $\bar{x}\pm s$)

Group	Cell count of invasion (/field)	Cell count of migration (/field)
Control	96.12±4.23	108.65±7.18
Curcumin treated	44.51±6.12*	68.74 ± 6.53*

*P<0.05, vs Control group

Effects of Curcumin on the Expressions of MMP-2, MT1-MMP and NF-κB

As shown in Figure 2 and Table 2, after being treated with 50μmol/L curcumin for 72h, the expressions of MMP-2, MT1-MMP and NF-κB were all decreased (P<0.05).

Table 2. Effects of curcumin on the expressions of MMP-2, MT1-MMP and NF-κB in Caski cells
($\bar{x}\pm s$, n=3)

Group	MMP-2/ β-action	MT1-MMP/ β-action	NF-κB/ β-action
control	0.989±0.013	0.865±0.031	0.987±0.014
Curcumin treated	0.676±0.033*	0.471±0.038*	0.516±0.112*

*P<0.05 vs control group

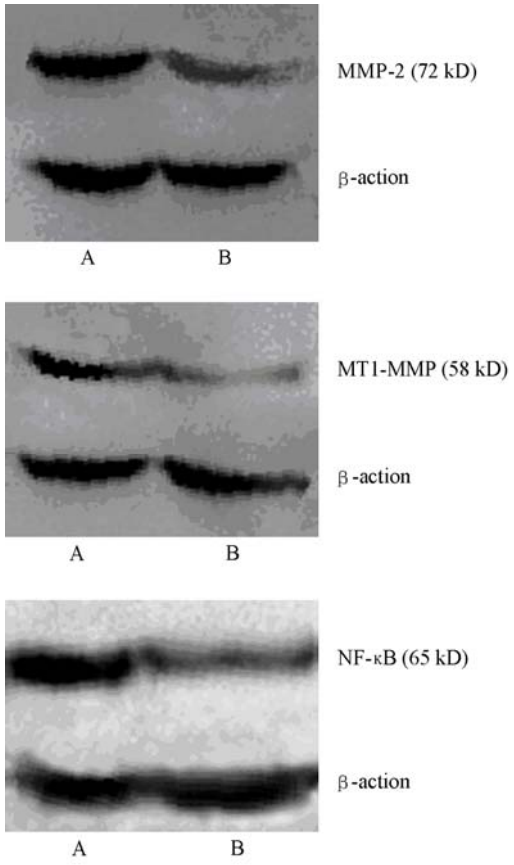

Figure 2. Expressions of MMP-2, MT1-MMP and NF-κB protein in Caski cells.
A: Curcumin treated group; B: Control group

DISCUSSION

Curcumin has been widely studied for its tumour inhibiting properties. It has been reported that curcumin can inhibit the proliferation of tumor cells and induce apoptosis through the multiple pathways and tumor-related factors. In our previous report, we have analyzed the effects of curcumin on proliferation and apoptosis of human cervical cancer *in vitro* and *in vivo*[8,9]. In this study, we observed that after being treated with curcumin, the proliferation of human cervical cancer cell line Caski was inhibited in a time and dose-depending manner and the invasion and metastasis were significantly inhibited. We have further explored the possible mechanism.

Matrixmetalloproteinases (MMPs) are a family of zinc-dependant endopeptidases which can degrade components of the extracellular matrix (ECM)[10,11] allowing tumor cells to migrate to their secondary sites of growth via blood and lymphatic vessels. MMP-2 (also known as Gelatinase A) is a 72 kDa. Type IV collagenase which degrades various ECM

components like gelatins, collagens and elastin. It is known that highly metastatic cells become less aggressive when MMP-2 expression or activity is reduced[12,13] and previous studies have also shown that curcumin reduces MMP-2 expression in breast carcinoma cell lines[14]. This reduction of MMP-2 activity could be an important reason for anti-metastatic property of curcumin. In our study, we have demonstrated that the expression level of MMP-2 was significantly decreased.

MMP-2 is activated at the tumor cell surface by an unique pathway involving an 'activation complex' composed of membrane type-1 matrix-metalloproteinase (MT1-MMP), tissue inhibitor of matrixmetallo-proteinase-2 (TIMP-2) and pro-MMP-2 with the help of αvβ3 integrin receptor, and MT1-MMPs play a dual role in cell surface proteolysis which cleave ECM components and also activate secreted MMP-2. Thus downregulation of MT1-MMP on treatment of Caski cells with curcumin may reduced MMP-2 activity.

NF-κB is a family of transcription factors, which have been known to be involved in the control of a large number of normal cellular processes such as inflammatory and immune responses, cell growth and apoptosis. NF-κB inducible genes play an important role in various disorders, especially cancer. NF-κB induces antiapoptotic genes and protects cancer cells from apoptosis[15]. They also induce the expression and activation of MMPs, which play a role in ECM degradation and facilitate cell motility and tumor growth[16]. There have been reports that curcumin inhibits NF-κB activation induced by several agents[17]. In our study, we also observed that the expression of NF-κB was significantly decreased.

Taken together, curcumin could inhibit the invasion and metastasis of human cervical cell Caski. It is speculated that down-regulating the expressions of MMP-2 and MT1-MMP is probably one of the mechanisms. We also think that the inhibiting effect of curcumin on MMPs may be mediated by down-regulation of the expression of NF-κB.

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