

Full Length Research Paper

# Curcumin inhibits invasion and metastasis in the human ovarian cancer cells SKOV3 by CXCL12–CXCR4 axis

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**Effects of curcumin on invasion and metastasis in the human ovarian cancer cells SKOV3 and approach if this inhibitory effects are related with CXCL12–CXCR4 axis were carefully studied. SKOV3 cells were treated with 10, 25, 50  $\mu\text{mol/l}$  curcumin for 24, 48 and 72 h. Proliferation of SKOV3 cells was measured with 3-(4,5-demethy-2-thiazolyl)-2,5- dephenyl-2H-tetrazolium-bromide (MTT) assay. Invasion and metastasis of SKOV3 cells were evaluated with transwell chamber. The expressions of CXCL-12 and CXCR4 were detected by western-blot. After being treated with curcumin, the proliferation of SKOV3 cells was inhibited in a time and dose-dependent manner ( $P < 0.05$ ). In invasion assay, the number of cells in curcumin treated group to migrate to filter coated with Matrigel was reduced compared with the 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 the control group ( $P < 0.05$ ). The expression level of CXCL-12 and CXCR4 were decreased. Our study indicated that curcumin could inhibit the invasion and metastasis of the human ovarian cancer cells SKOV3. Inhibiting the expression of CXCL-12 and CXCR4 was probably one of its molecular mechanisms.**

**Key words:** Ovarian cancer, curcumin, invasion, metastasis.

## INTRODUCTION

Curcumin, a diferuloylmethane, is a major active component of the food flavor turmeric (*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 been shown to display anticarcinogenic (Balasubramanian and Eckert, 2007), antioxidant (Zhao et al., 2008), anti-inflammation (Guo et al., 2008) and antiangiogenic properties (Bhandarkar and Arbiser, 2007) as well as modulation of multidrug-resistance gene and protein function (Choi et al., 2008). In recent years, it has also been reported that curcumin reduced cancer cells invasion *in vitro* and *in vivo* (Menon et al., 1999; Chen et al., 2004). But the mechanisms remain unclear.

For most cancers, morbidity and eventual mortality result from metastasis (Liotta, 2001). The metastasis of ovarian cancer is different from that of the other types of

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**Abbreviations:** RPMI, Roswell Park Memorial Institute; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; HRP, horseradish peroxidase; IgG, immunoglobulin G; MMP-2, matrix metalloproteinase-2; MT1-MMP, membrane type-1 matrix metalloproteinase; NF- $\kappa$ B, nuclear factor kappa B; SDF1, stromal cell-derived factor 1.

epithelial tumors (Naora and Montell, 2005). At its earliest stage, the tumor is limited to one or both ovaries with an intact ovarian capsule. Once the capsule is disrupted, the tumor cells may directly invade the adjacent tissues including the uterus, fallopian tubes, peritoneum and the broad ligament; or implant into the peritoneum, pelvic methothelium, or abdominal organs. Metastasis of various cancers to distant organs is not a random event, but is a regulated, site-specific process (Chambers et al, 2002; Pantel and Brakenhoff, 2004). Many chemokine and their receptors are detectable in various cancers (Backwill and Mantovani, 2001). In breast and thyroid cancers, chemokine receptors CXCR4 and its ligand CXCL12 have been shown to be involved in metastasis (Muller et al., 2001; Kryczek et al., 2007). CXCR4 is expressed in many types of cancer cells, including epithelial, mesenchymal, and hematopoietic tumor cells (Backwill, 2004). Clinical studies also found that the prognosis of patients with a high level of CXCR4 is significantly worse than that of the patients with a low CXCR4 level (Russell et al., 2004; Kaifi et al., 2005; Kim et al., 2005), indicating that CXCL12-CXCR4 axis plays an important role in the behavior of tumor. In this study, we firstly examined the effect of curcumin on the expressions of CXCL12 and CXCR4 and the invasion and metastasis of the human ovary cancer cells SKOV3 *in vitro*.

## MATERIALS AND METHODS

### Materials

SKOV3 cell line was supplied by the Department of Pathophysiology of Chong qing medical University. Curcumin and 3-(4,5-dimethyl-2-thiazolyl)-2,5-dephenyl-2H-tetrazolium-bromide (MTT) were obtained from Sigma (St Louis, Mo, USA). Roswell Park Memorial Institute (RPMI)-1640 and fetal bovine serum (FBS) from Hyclone. The mouse monoclonal anti-CXCL-12 and anti-CXCR4 antibodies were purchased from Abcam (USA).

### Cells culture

SKOV3 cells were maintained in RPMI-1640 containing 10% FBS and antibiotic-antimycotic. The cells were cultured in a humidified incubator in 5% CO<sub>2</sub> at 37°C in air.

### MTT assay

Cell growth was measured by a modified MTT assay. About  $1 \times 10^5$  cells /well were plated in 96-well microplates and incubated overnight. Cells were then treated with 10, 25 and 50  $\mu\text{mol/l}$  curcumin for 24, 48 and 72 h. Then 20  $\mu\text{l}$  stock MTT was added to each well and the cells were further incubated at 37°C for 4 h. The supernatant was removed and 200  $\mu\text{l}$  dimethyl sulfoxide (DMSO) in isopropanol was added to each well to solubilize the formazan produced. The absorbance at wavelength of 570 nm was measured by a micro enzyme-linked immunosorbent assay (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

SKOV3 cells were treated with 50  $\mu\text{mol/l}$  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 the filter was coated with Matrigel (BD, USA). Lower compartment was filled with supernatant 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  $\times 400$ . 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

SKOV3 cells were treated with 50  $\mu\text{mol/l}$  curcumin for 72 h. After culture solution was discarded, the cells of control group and treated group collected were about  $5 \times 10^6$ . The solution (containing 0.1 mol/l NaCl, 0.01 mol/l Tris HCl, Ph7.6, 0.001 mol/l ethylenediaminetetraacetic acid (EDTA), 100 g/ml phenylmethanesulfonylfluoride (PMSF) and 2  $\mu\text{g/ml}$  Leupeptin) was used to lyse the cells for 30 min. After centrifugation at 10000 r/min for 10 min, the supernatant was collected. All above-mentioned operations were done at 4°C. Protein concentration was measured and samples with 25  $\mu\text{g}$  protein were loaded and run on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred onto nitrocellulose membrane. The membrane was blocked with 5% fat-free milk for 1 h, then probed with antibodies against CXCL-12 and CXCR4 (1:1000) and beta-actin (1:1000) and then 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 immunoglobulin G (IgG) (1:1000) for 2 h 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.

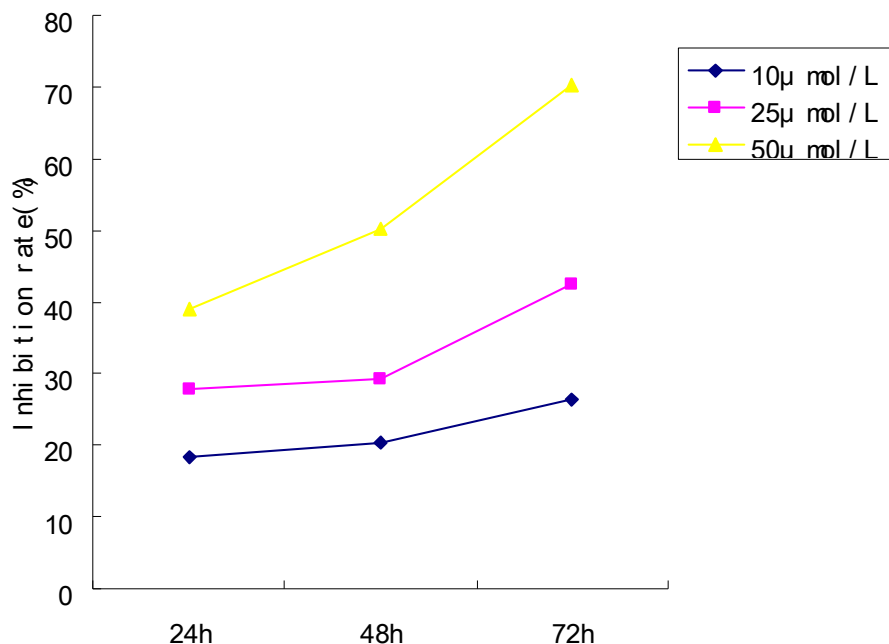
### Statistical analysis

All data are expressed as mean  $\pm$  SEM. Statistical differences between the various groups were assessed with a one-way analysis of variance (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 SKOV3 cells by MTT

SKOV3 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.



**Figure 1.** Inhibition of curcumin on SKOV3 cells growth.

**Table 1.** Effects of curcumin on invasion and migration of SKOV3 cells (% ,  $n = 9$ ,  $\bar{x} \pm s$ ).

Treatment	Cell count of invasion (/field)	Cell count of migration (/field)
Control	102.31 $\pm$ 7.22	120.76 $\pm$ 8.15
Curcumin	51.14 $\pm$ 3.12*	73.54 $\pm$ 4.43*

\*P < 0.05 , vs Control group.

The results showed great differences between the control group and the treated groups (P<0.05, Figure1).

#### Effects of curcumin on invasion and migration of SKOV3 cells

As can be seen in Table1, after being treated with 50  $\mu$ mol/l curcumin, the invasion and migration of SKOV3 cells were significantly decreased. In invasion assay, the number of cells in curcumin treated group (102.31  $\pm$  7.22) to migrate to filter coated with Matrigel was significantly reduced compared with control group (51.14  $\pm$  3.12) (P<0.05). Meanwhile, in migration assay, the number of cells in curcumin treated group (120.76  $\pm$  8.15/field) to migrate to filter was also decreased compared with control group (73.54  $\pm$  4.43/field) (P<0.05).

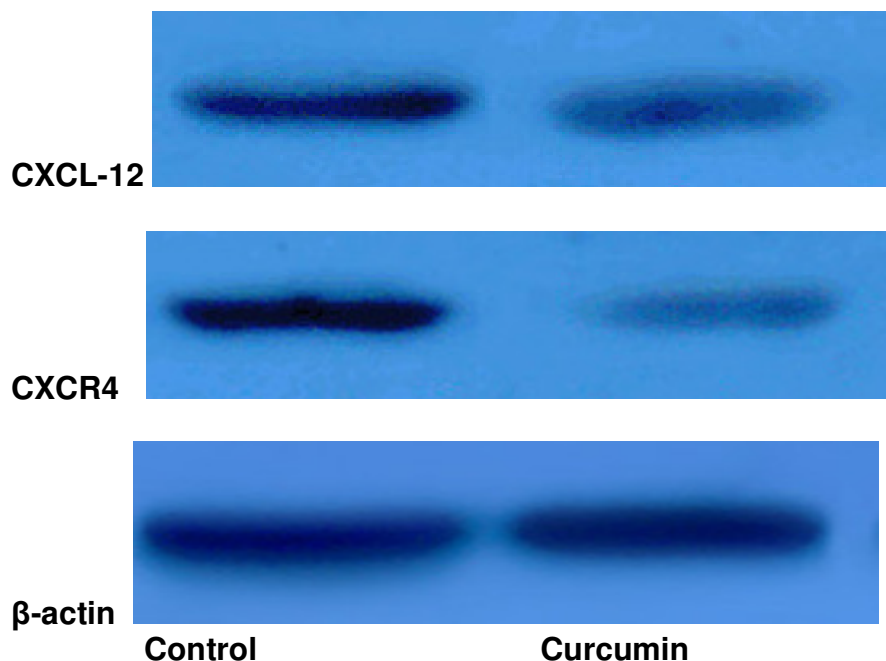
#### Effects of curcumin on the expression of CXCL-12 and CXCR4

As shown in Figure 2 and Table 2, after being treated with

50  $\mu$ mol/l curcumin for 72 h, the expression of CXCL-12 (0.304  $\pm$  0.013) was decreased compared with control group (0.683  $\pm$  0.014). Meanwhile, the expression of CXCR4 in curcumin treated group (0.906  $\pm$  0.031) was significantly decreased compared with control group (0.211  $\pm$  0.028) (P<0.05).

#### 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 analyzed the effect of curcumin on proliferation and inducing apoptosis of human cervical cancer *in vitro* and *in vivo* (Zhao et al., 2004, 2007). We also found that curcumin could inhibit the invasion and metastasis of human cervical cells Caski by down-regulating the expression of matrixmetalloproteinase-2 (MMP-2), membrane type-1 matrixmetalloproteinase (MT1-MMP) and nuclear factorkappa B (NF- $\kappa$ B) is one possible mechanism (Xu et al, 2009). In this study, we



**Figure 2.** Expression of CXCL-12 and CXCR4 protein in SKOV3 cells.

**Table 2.** Effects of curcumin on expression of CXCL-12 and CXCR4 in SKOV3 cells ( $\bar{x} \pm s$ ,  $n = 3$ ).

Group	CXCL-12 / $\beta$ -action	CXCR4 / $\beta$ -action
control	0.683 $\pm$ 0.014	0.906 $\pm$ 0.031
Curcumin treated	0.304 $\pm$ 0.013*	0.211 $\pm$ 0.028*

\*P < 0.05 vs control group.

observed that after being treated with curcumin, the proliferation of human ovary cancer cell line SKOV3 was inhibited in a time and dose-depending manner and the invasion and metastasis were significantly also inhibited. We have further explored the possible mechanism.

Chemokines are small, secreted peptides that control adhesion and transendothelial migration of leukocytes, lymphocytes and monocytes, especially during immune and inflammatory reactions. Chemokines combine with G-protein-coupled-receptors to perform their function. Chemokines and their receptors have significant roles in tumor metastasis, recurrence and angiogenesis (Balkwill, 2004). Among the family of chemokines and their receptors that significantly contribute to the initiation and development of tumors, CXCL12 and its receptor CXCR4 are the most frequently mentioned. CXCR4 is the unique receptor for CXCL12, also named stromal cell-derived factor 1 (SDF1). Tumor cells expressing high CXCR4 are often found in tissue expressing CXCL12, perhaps due to organ-specific metastasis (Shirozu et al., 1995). One possible mechanism involves chemokines combining with their receptors to induce membrane wrinkling in tumor

cells that forms pseudopodia. The tumor cells then adhere to and traverse through the extracellular matrix and basal membrane to enter the blood circulation and metastasize. Furthermore, previous views suggested that tumor cells express high CXCR4 and the metastasis-targeted organs express high CXCL12, so the tumor cells were attracted to the ligand in these organs. Previous study has also shown that curcumin has an inhibitory effect on CXCR4-induced human retinal endothelial cells migration (Zaheer et al., 2008). This reduction of CXCL12- CXCR4 activity could be an important reason for anti-metastatic property of curcumin. In our study, we have demonstrated that the expression level of CXCL-12 and CXCR4 was significantly decreased after being treated with curcumin.

Nuclear factor kappa B (NF- $\kappa$ B) is a family of transcription factors, which have been known to be involved in the control of large number of normal cellular processes such as inflammatory and immune responses, cell growth and apoptosis. NF- $\kappa$ B inducible genes play an important role in various disorders, especially cancer. NF- $\kappa$ B induces antiapoptotic genes and protects cancer cells

from apoptosis (Beg and Baltimore, 1996). A recent study suggested that NF- $\kappa$ B is a potent inducer of cell surface CXCR4 (Mabuchi et al., 2004). Tumor cells in omental metastases seemed to up-regulate CXCR4 expression easily through cytokines produced in lymphoid nodule and then the activation of NF- $\kappa$ B. It may cause the trend that the rate of CXCR4 expression (77%) in omental metastases was higher than primary tumors (59%). There have been reports that curcumin inhibits NF- $\kappa$ B activation induced by several agents (Brennan and O'Neill, 1998). In our previous study (Xu et al., 2009), we also observed that curcumin could significantly inhibit the invasion and metastasis of human cervical cancer cells, Caski, by down-regulating the expression of NF- $\kappa$ B. Combined with the results in this study, we supposed that curcumin reduced ovarian cancer metastasis through NF- $\kappa$ B - mediated expression of CXCL12 and CXCR4.

In conclusion, curcumin could inhibit the invasion and metastasis of human ovarian cancer cells SKOV3. It was speculated that down-regulating the expression of CXCL-12 and CXCR4 is one possible mechanism. The inhibiting effect of curcumin on CXCL-12 and CXCR4 might be mediated by down-regulating the expression of NF- $\kappa$ B.

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