Inhibition of curcumin on human lung adenocarcinoma LTEP-A2 cells and its mechanism

Zhu Mingao and Chen Lei*
Department of Oncology, The second affiliated hospital of Soochow University, Suzhou, PR China

Abstract: This study was designed to explore the effects of curcumin on proliferation, migration and invasion of human lung adenocarcinoma LTEP-A2 cells and determine its mechanism. Human lung adenocarcinoma LTEP-A2 cell was cultured in vitro. After incubation with different concentrations of curcumin (5, 10, 15 μmol/L), the effects of curcumin on proliferation, migration and invasion of human lung adenocarcinoma LTEP-A2 cells were observed by MTT assay, wound healing and transwell assay. The expression levels of COX-2 and MMP-9 were detected by western blot. Compared with the blank control group, curcumin decreased the survival rate of LTEP-A2 cells, shorten the cell migration distance and decrease the number of LTEP-A2 cells penetrating membrane. The expression levels of COX-2 and MMP-9 were both down-regulated by curcumin. Curcumin can inhibit the proliferation, migration and invasion of human lung adenocarcinoma LTEP-A2 cells. The mechanism may be related to the down-regulation of COX-2 and MMP-9 expression.

Keywords: Curcumin, lung adenocarcinoma cell, cell proliferation.

INTRODUCTION

Non-small cell lung cancer (NSCLC) is often asymptomatic at the early stage, and the patient's disease is already in advanced stage at the time of treatment. Therefore, chemotherapy is the most comprehensive treatment (Schiller *et al*., 2002). Cisplatin has a broad spectrum of anti-tumor activity and is the first-line drug in NSCLC chemotherapy. However, with the development of chemotherapy in patients with advanced lung cancer, the problem of acquired drug resistance of cisplatin has become increasingly serious, which has restricted its efficacy (Giaccone *et al*., 2004; Fukuoka *et al*., 2016). Curcumin is a phenolic compound extracted from the rhizome of traditional Chinese medicine, such as turmeric, *Radix curcumae*, *Curcuma zedoary* and calamus. It has various functions, such as lowering blood pressure, anti-myocardial ischemia, anticoagulation and reducing blood lipid (Kunnumakkara *et al*., 2007; Notarbartolo *et al*., 2005). In recent years, in vitro experiments have shown that curcumin can inhibit the proliferation of a variety of tumor cells including colon cancer, prostate cancer, etc., and can also induce apoptosis of tumor cells (Yang *et al*., 2015; Milacic *et al*., 2008). However, there are few reports on whether curcumin can induce apoptosis of human lung cancer cells and its mechanism. In this study, the effects of curcumin on proliferation, migration and invasion of human lung adenocarcinoma LTEP-A2 cells, and the expression of COX-2, MMP-9 were observed so as to explore the anti-tumor effect and mechanism of curcumin on lung adenocarcinoma cells, thereby providing experimental basis for the study of high-effective and low-toxic traditional Chinese medicine anti-tumor drugs.

*Corresponding author: e-mail: tpudo144@aliyun.com

MATERIALS AND METHODS

Cell culture

Human lung adenocarcinoma LTEP-A2 cells (Cell bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences) were cultured in an MEM medium containing 10% fetal bovine serum (Gibco Company, USA) in a humidified 37°C, 5% CO_2 incubator.

Determination of proliferation of LTEP-A2 cells by MTT assay

LTEP-A2 cells in logarithmic growth phase were seeded into 96-well plates at a density of 2×10^4 cells/mL. After adherence for 24 hours, LTEP-A2 cells were incubated with different concentrations of curcumin (5, 10, 15 μmol/L) (purchased from Sigma company) and temozolomide, which served as positive control (10 μmol / L) (Sigma company) for 72h, respectively. MTT solution was added to make the final concentration of 0.5mg/mL. After 4 hours of culture, the solution in the hole was discarded, and the blue-purple complex was dissolved by adding 100 mL dimethyl sulfoxide (DMSO), and then shaken for 10 minutes. The absorbance was measured at wavelength of 490 nm by enzyme labeling instrument (A_{490}). Cell viability = treatment group (A_{490}) / control group (A_{490}) × 100%

Wound healing for detecting migration of LTEP-A2 cells

The LTEP-A2 cells in the logarithmic growth phase were inoculated in a 96-well plate at a density of 5×10^4 cells/mL. After the cells were attached, a sterile 100mL micropipette tip was used to scribe horizontally in the cell layer to form a uniform width of wounds, resulting in a cell wound model, photographed under a microscope and

the wounds widths were determined. The wounds widths were measured after 24 hours. The wound area was quantified by Image J software, three measurements are taken for each wound and the average width is calculated.

Transwell assay for detecting the invasion of LTEP-A2 cells

The invasion model was constructed by transwell chamber method. The concentration of LTEP-A2 cells was adjusted to 5×10^5 cells/mL with serum-free MEM. 0.25mL of above liquid was added to the transwell upper chamber and drug intervention was performed (The curcumin concentration was 10μmol/L, and the positive control group was 10μmol/L of temozolomide.). Then 0.5 mL of MEM containing 10% fetal bovine serum was added to the lower chamber. After incubating in a cell culture incubator for 72 hours, the chamber was taken out and the liquid in the chamber was discarded. The chamber was stained in 0.1% crystal violet staining solution for 20 min. After washing with pure water, the cells on the upper surface of the chamber filter were wiped with a cotton swab. After drying, it was decolorized with 200μL of acetic acid decolorizing solution for 15 min and 100μL of the above solution was placed on a 96-well plate to measure the OD value at wavelength of 560 nm.

Western blot analysis of protein expression of COX-2 and MMP-9

After treatment with curcumin (10μmol/L), LTEP-A2 cells were lysed to extract total cellular protein, and the protein concentration was determined using a BCA protein assay kit. 30μg of protein was subjected to 10% SDS-PAGE electrophoresis to separate proteins. After transferred to PVDF membrane, the cells were blocked with 5% skim milk powder for 1h and then rabbit anti-human COX-2 and MMP-9 monoclonal antibodies (Cell signaling Company, 1: 1000) were added and incubated at 4^oC overnight. HRP-labeled goat anti-rabbit secondary antibody (Shanghai Kangcheng Company) was incubated for 2 hours and was finally analyzed by X-ray film exposure. β-actin was used as internal control (Shanghai Kangcheng Company).

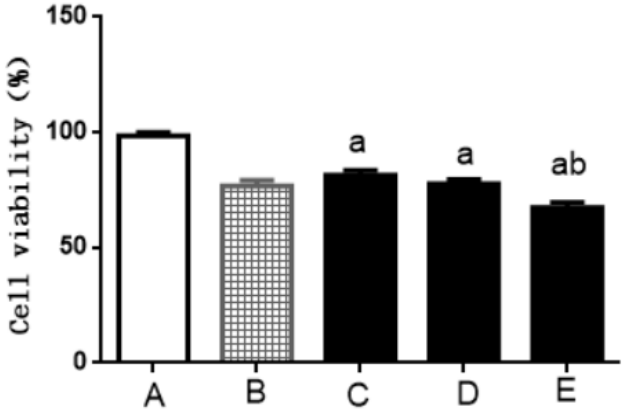
STATISTICAL ANALYSIS

SPSS 25.0 statistical software was used for data analysis. The data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The comparison between groups was performed by One-way ANOVA and the Tukey method was used for comparison between groups. P<0.05 value was considered statistically significant.

RESULTS

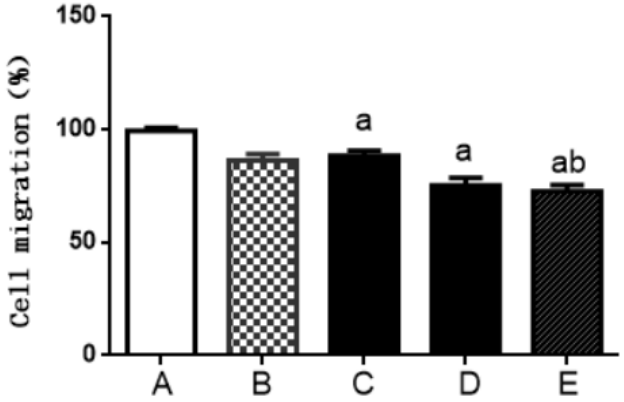
Effect of curcumin on proliferation of LTEP-A2 cells

MTT assay showed that different concentrations of curcumin (5, 10, 15μmol/L) significantly reduced the survival rate of LTEP-A2 cells and inhibited the proliferation of LTEP-A2 cells in a concentration-dependent manner when compared with the blank control group. Compared with temozolomide in the positive control group, high concentration of curcumin (15 μmol/L) had a strong inhibitory effect on LTEP-A2 cells, and the difference was statistically significant (P<0.05) (fig. 1).



Compared with group A, [a]P<0.05; compared with group B, [b]P<0.05.
A: blank control group; B: temozolomide of positive control group; C: 5 μmol/L of curcumin; D: 10μmol/L of curcumin; E: 15 μmol/L of curcumin

Fig. 1: Effect of curcumin on proliferation of LTEP-A2 cells



Compared with group A, [a]P<0.05; compared with group B, [b]P<0.05.
A: blank control group; B: temozolomide of positive control group; C: 5 μmol/L of curcumin; D: 10 μmol/L of curcumin; E: 15 μmol/L of curcumin

Fig. 2: Effect of curcumin on migration of LTEP-A2 cells

Effect of curcumin on migration of LTEP-A2 cells

The effect of curcumin on the migration ability of LTEP-A2 cells was observed by *in vitro* wound healing. The width of the wounds became smaller due to the migration of cells, and gradually merged. The results showed that compared with the blank control group, the cell migration distance of the curcumin group (5, 10, 15μmol/L)

decreased (P<0.05), and the greater the curcumin concentration, the smaller the migration distance of the LTEP-A2 cells (P<0.05) (fig. 2), indicating that curcumin can inhibit the migration of LTEP-A2 cells.

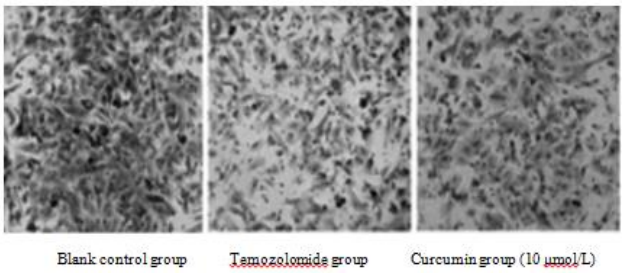
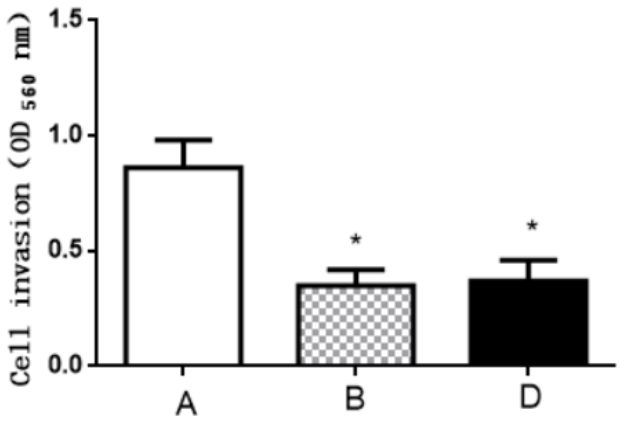


Fig. 3: Invasive cells stained with 0.1% crystal violet (200 X)



Compared with group A, *P<0.05
A: blank control group; B: temozolomide of positive control group; D: 10 μmol/L of curcumin;

Fig. 4: Comparison of OD_{560} nm after decolorization of acetic acid decolorizing solution

Effect of curcumin on invasion of LTEP-A2 cells

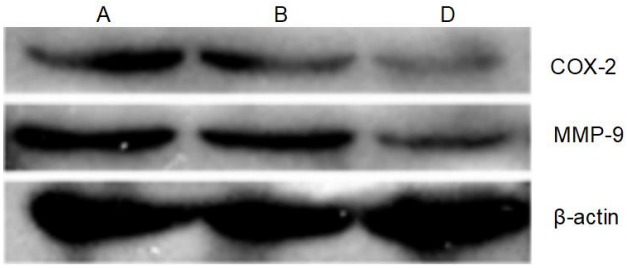
The results of transwell assay showed that the curcumin group (10 μmol/L) was similar to the temozolomide of positive control group and the OD value at the wavelength of 560 nm was significantly lower than that of the blank control group, suggesting that the number of LTEP-A2 cells passing through the transwell chamber filter was significantly reduced (figs. 3-4), indicating that curcumin can suppress the invasion ability of LTEP-A2 cells.

Effect of curcumin on the expression of COX-2 and MMP-9 proteins in LTEP-A2 cells

Western blot results showed that COX-2 and MMP-9 protein expression in LTEP-A2 cells were down-regulated after curcumin treatment as shown in fig. 4, which was similar to that of temozolomide in the positive control group, suggesting that curcumin's inhibition on LTEP-A2 cells might be related to the expression of COX-2 and MMP-9 invasion-related proteins.

LTEP-A2 cells

A: blank control group; B: temozolomide of positive control group; D: 10μmol/L of curcumin;



A: blank control group; B: temozolomide of positive control group; D: 10 μmol/L of curcumin;

Fig. 5: Effect of curcumin on the expression of COX-2 and MMP-9 proteins in LTEP-A2 cells

DISCUSSION

Lung cancer is one of the most common malignant tumors in the world. Invasion and metastasis are the most essential biological characteristics and the root cause of death in lung cancer patients (Milacic *et al*., 2008; Liang *et al*., 2015). Turmeric is a traditional Chinese medicine in China, derived from the roots of the genus Curcuma. Modern medical research (Hoang *et al*., 2015) has confirmed that the main active ingredient in turmeric is curcumin and volatile oil, which has anti-inflammatory, anti-oxidation, hypolipidemic and anti-tumor effects. In recent years, more studies have shown that curcumin has the effect of inhibiting tumors, such as gastric cancer, liver cancer, breast cancer, etc (Thulasiraman *et al*., 2014; Verma *et al*., 1997; Kazemilomedasht *et al*., 2013).

However, most of the related studies focus on the inhibition of tumor angiogenesis by curcumin. For example, studies have shown that curcumin can inhibit tumor angiogenesis by inhibiting proliferation and migration of endothelial cell. However, there is little research on the inhibitory effect of curcumin on tumor cells themselves. In this study, the inhibition of curcumin on tumor was confirmed in human lung adenocarcinoma LTEP-A2 cells. The results showed that a certain concentration of curcumin can evidently inhibit the proliferation of LTEP-A2 cells in a concentration-dependent manner. Compared with the temozolomide in positive control group, the inhibitory effect of curcumin on LTEP-A2 cells is not weaker than temozolomide.

High invasiveness of lung adenocarcinoma is an important reason for high recurrence of lung adenocarcinoma and cell migration and invasiveness are the key factors affecting cell invasiveness. Studies (Lin *et al*., 2009) have shown that certain Chinese patent medicine can exert anti-tumor effects by inhibiting the migration and invasion of cancer cells. In this study, the

wound healing and transwell invasion test indicated that curcumin can effectively inhibit the migration and invasion of LTEP-A2 cells. As the concentration increased, the effect of curcumin on the migration of LTEP-A2 cells was gradually enhanced.

Cyclooxygenase (COX) is a rate-limiting enzyme that catalyzes the synthesis of prostaglandins from arachidonic acid, including two subtypes, COX-1 and COX-2. COX-2 is an inducible immediate response gene, which is normally present only in the kidney, brain tissue and placenta in late pregnancy. When stimulated by inflammatory mediators or cancer-promoting factors, its expression is up-regulated and is closely related to lymph node metastasis, differentiation and depth of invasion of various tumors such as colon cancer and lung cancer (Liu *et al*., 2008). MMPs are a class of zinc-dependent proteases that degrade extra cellular matrix proteins. MMP-9 is a gelatinase in MMPs that degrades the major components of the perivascular basement membrane, such as type IV collagen, laminin and fibronectin, which facilitates the invasion of tumor cells along the basement membrane into surrounding tissues (Yang *et al.,* 2015). Studies (Zhang *et al.,* 2017) have shown that the high invasiveness of lung adenocarcinoma is associated with the expression of COX-2 and MMP-9. The results of this study showed that curcumin can significantly down-regulate the protein expression of COX-2 and MMP-9 in LTEP-A2 cells, indicating that the inhibition of curcumin on human lung adenocarcinoma LTEP-A2 cells may be related to down-regulation of the expression of two invasive proteins, COX-2 and MMP-9.

In summary, as a monomeric component of traditional Chinese medicine, curcumin inhibits the proliferation, migration and invasion of human lung adenocarcinoma cell line LTEP-A2. The mechanism of inhibition may be related to the down-regulation of COX-2 and MMP caused by curcumin.

CONCLUSION

The results of this study provide an experimental basis for further investigation on the mechanism of curcumin's inhibition on lung adenocarcinoma and for clinical exploration of curcumin as a therapeutic target for lung adenocarcinoma. At the same time, it also provides a new idea for searching for effective and low toxic components of traditional Chinese medicine (TCM).

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