JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2017 June 15; 37(3): 355-360
ISSN 0255-2922

RESEARCH ARTICLE

Curcumin co-treatment ameliorates resistance to gefitinib in drug-resistant NCI-H1975 lung cancer cells

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Supported by Natural Science Found of Zhejiang Province: Research of Molecular Mechanism of Curcumin Reversing TKI Targeted Drug Resistance of NSCLC (No. LY13H160037)

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Accepted: February 22, 2016

Abstract

OBJECTIVE: To examine whether a combinative treatment with curcumin enhances the effects of the epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib on cell proliferation, clonogenic capacity and apoptosis in the drug-resistant lung cancer cell line NCI-H1975, and further investigate the molecular mechanisms involved.

METHODS: NCI-H1975 cells were treated with curcumin and gefitinib alone or in combination, and cell proliferation, clonogenic capacity and apoptosis were examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, clone forming experiments, and flow cytometry, respectively, while p38, extracellular regulated protein kinase (ERK)1/2, and protein kinase B (AKT) phosphorylation were examined using Western blotting.

RESULTS: Compared with the effects of either agent alone, the combination of curcumin and gefitinib had a stronger suppressive effect on proliferation and the clonogenic capacity ($P < 0.05$), and showed an increased ability to promote apoptosis ($P < 0.05$) and reduce p38, ERK1/2, and AKT phosphorylation ($P < 0.05$).

CONCLUSION: Co-treatment of curcumin and gefitinib significantly improves the ability of gefitinib to inhibit cell proliferation, suppress the clonogenic capacity and enhance apoptosis in NCI-H1975 cells, and these effects are possibly mediated *via* a decrease in phosphorylation of proteins in downstream pathways of the epidermal growth factor receptor.

Keywords: Lung neoplasms; Cell line, tumor; Curcumin; Cell proliferation; Apoptosis; Receptor, epidermal growth factor; Gefitinib

INTRODUCTION

Lung cancer is one of the most common malignant tu-

mors and is a major cause of cancer-related death.[1] Non-small cell lung cancer (NSCLC) accounts for about 80% of lung cancer cases, and traditional chemotherapy is of limited effect in its treatment. With the development of molecular biology techniques and the study of the pathogenesis of molecular mediators in lung cancer, molecular targeted therapy is now the most promising research strategy for the treatment of advanced NSCLC, and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib has been proven efficacious in the targeted treatment of NSCLCs.[2]

However, recent studies show that even patients who are highly sensitive to EGFR-TKIs may become resistant to these drugs (acquired resistance) after a median survival period of 7-9 months as the disease progresses.[3] At present, there is no standard treatment for this kind of acquired resistance, and efforts are ongoing to identify drug combinations that can combat this issue.

Curcumin is a phenolic pigment extracted from a perennial herb of the Zingiberaceae family, Jianghuang (*Rhizoma Curcumae Longae*) It has anti-inflammatory, antitumor, and antioxidant properties with the advantage of having very little toxic-side effects.[4] Curcumin can inhibit the proliferation of a variety of tumors, including lung cancer, liver cancer, breast cancer and colon cancer in vitro, and induce tumor cell apoptosis.[5]

The purpose of this study was to investigate whether a combinatorial treatment with curcumin can enhance the effects of gefitinib on cell proliferation, clone formation and apoptosis, using an EGFR-TKI drug-resistant strain of NCI-H1975 cells *in vitro*, and probe into the molecular mechanisms.

METHODS

Reagents and instruments

Gefitinib and curcumin were obtained from Sigma-Aldrich (St. Louis, MO, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and RPMI 1640 from Life Technologies, Inc., (Carlsbad, CA, USA); Annexin V-FITC from Nanjing KeyGen Biotech. Co., Ltd., (Nanjing, China); Antibodies against P-P38, P-ERK1/2 , P-AKT from Cell Signaling (Danvers, MA, USA).

Cell culture

The human NSCLC cell line NCI-H1975 was provided by the Zhejiang Provincial Academy of Medical Sciences (Hangzhou, China), and was cultured in RPMI 1640 supplemented with 10% FBS at 37 ℃ in a CO_2 constant temperature incubator (Thermo Forma 3121; Thermo Scientific, Waltham, MA, USA) at 100% saturated humidity and 5% CO_2 When the cell fusion rate reached about 80%, the cells in the logarithmic growth phase were collected under sterile conditions, and sub-cultured following trypsin digestion.

Determination of drug concentration and grouping

First of all, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the effects of curcumin or gefitinib on NCI-H1975 and to determine each IC_{50} (half maximal inhibitory concentration) index. Use IC_{15} as working concentration of curcumin, which was 10 ng/mL, and select IC_{50} as working concentration of gefitinib, which was 0.1 μmol/L.

The four groups were set up. control group: cells treated without curcumin or gefitinib; curcumin treatment group): cells treated with curcumin at 10 ng/mL; gefitinib treatment group: cells treated with gefitinib at 0.1 μmol/L; curcumin + gefitinib treatment group: cells treated with gefitinb at 0.1 μmol/L and curcumin at 10 ng/mL.

MTT assay

NCI-H1975 cells in the logarithmic growth phase were seeded in 96-well plates at a density of 5×10^3 cells/well, with 100-μL aliquots added per well. Preliminary experiments revealed that the gefitinib IC_{50} was 0.1 μmol/L and the curcumin IC_{15} was 10 ng/mL, which were used to treat the cells with gefitinib and curcumin alone or in combination. MTT assay was conducted as per the manufacturer instructions in triplicate, and the optical density (OD) at 570 nm was read using a desktop microplate reader (BIO-RAD 680; BIO-RAD, Hercules, CA, USA). The cell survival rate was calculated as follows:

Survival rate (%) = [OD (Experimental group)/OD (control group)] × 100(%).

Apoptosis detection by flow cytometry

NCI-H1975 cells in the logarithmic growth phase were grown in a culture flash for 24 h, and then treated with the agents (curcumin at 10 ng/mL and gefitinib at 0.1 mol/L, alone or in combination, or vehicles alone), and cultured for another 24 h. The cells were then detached using 0.25% trypsin and the resultant cell suspension was washed to remove trypsin, centrifuged at 1000 rpm for 5 min, and washed 3 times using ice-cold PBS. The cell pellet was then gently resuspended in the binding buffer, transferred into a 5-mL flow tube; and the cells were then incubated with Annexin V-FITC for 15 min at room temperature in the dark, rinsed once using the binding buffer, and resuspended with propidium iodide (PI) and incubated in the dark for 5 min. The frequency of cells in early or late apoptosis was then analyzed after collecting 10 000 cells/sample using the Beckman Coulter EPICS-XL system (Beckman Coulter, Inc., Brea, CA, USA): Live cells were Annexin V^-/PI^-, those undergoing early apoptosis were Annexin V^+/PI^-, and those in middle stage or advanced apoptosis were Annexin V^+/PI^+.

Colony-forming assay

A cell counter was used to count the cells in the logarithmic growth phase. Cells were suspended in RPMI