RESEARCH ARTICLE

Curcumin Inhibits Human Non-small Cell Lung Cancer A549 Cell Proliferation Through Regulation of Bcl-2/Bax and Cytochrome C

Yue Li¹, Shuai Zhang¹, Jian-Xiong Geng¹, Xiao-Yang Hu²*

Abstract

We intended to study the mechanism of the inhibitory action of curcumin on human non-small cell lung cancer A549 cell. The cell growth was determined by CCK-8 assay, and the results indicated that curcumin inhibited the cell proliferation in a concentration dependent manner. And to further confirm the relative anti-cancer mechanism of curcumin, RT-PCR was carried out to analysis the expression of relative apoptotic proteins Bax, Bcl-2. We found that curcumin could up-regulate the expression of Bax but down-regulate the expression of Bcl-2 in A549 cells. In addition, curcumin affect the mitochondrial apoptosis pathway. These results suggested that curcumin inhibited cancer cell growth through the regulation of Bcl-2/Bax and affect the mitochondrial apoptosis pathway.

Keywords: Curcumin - Bcl-2 - Bax - cytochrome C - apoptosis - NSCLC cells

Introduction

Lung cancer has the highest incidence and mortality rate of all malignancies. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers, the majority of which are at an advanced stage and unresectable when diagnosed (Parkin et al., 2005). Surgery is the most common treatment given with curative intent. Other treatments including radiotherapy, combined chemoradiotherapy and adjuvant chemotherapy are commonly used as well (NCCC, 2011). However, the side effects of the radiotherapy or chemotherapy promote much work on the new effective medicine in treating NSCLC. Traditional Chinese Medicine (TCM) is widely used for cancer treatment in China. Many support its use in treatment for cancer, yet scientific evidence for the effect of TCM needs to be established.

Curcumin, the principal polyphenolic cucuminoid, extracted from the turmeric rhizome Curcuma longa Linn, has been vastly reported for its biological activities, including anti-inflammatory (Schaffer et al., 2011), anti-oxidant (Speciale et al., 2011), anti-infection (Na et al., 2011) and anti-cancer (Bansal et al., 2011). Moreover, curcumin can cross the blood-brain barrier and is neuroprotective in neurological disorders (Song et al., 2012). Recently, interest in studying the anti-tumor mechanism of curcumin seems to be mounting for it is effective and safe. Curcumin may be able to modulate multiple cellular pathways involved in carcinogenesis and thus behaves as a multi-targeted drug. The mechanisms involved including cell proliferation, cell cycle, migration, invasion and angiogenesis though suppression of the Janus kinase-STAT3 (Yang et al., 2012), the upregulation of α-antitrypsin (Xu et al., 2012) and HIF-1α mechanisms (Wang et al., 2013).

In this paper, we try to verify the anti-cancer mechanism of curcumin in the A549 cells of NSCLC. Therefore, we carried out RT-PCR and Western blot assay to evaluate the expression of Bcl-2, Bax and cytochrome C, which are important factors involved in cancer cell.

Materials and Methods

Materials

In this study, we prepared the following materials: curcumin (Sigma-Aldrich, St. Louis, MO), β-actin antibody (Sigma), cytochrome C monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), real-time polymerase chain reaction (RT-PCR) kit (GIBCO, Grand Island, NY, USA); Amplification of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) upstream and downstream primers were synthesized by Shanghai Shenggong Biological Engineering Technology Service Company (Shanghai, China).

Cell culture and treatment

Human NSCLC A549 cells were purchased from Shanghai cell bank of the Chinese Academy of Sciences

¹Department of Medical Oncology, The Third Affiliated Hospital of Harbin Medical University, Staff Room of Prescription, Heilongjiang University of Chinese Medicine, Harbin, China  *For correspondence: pda1981@163.com
Cells were treated with 5, 10, 20, 40 μmol/L curcumin (reference group with 0.5% DMSO) for 48 h, then washed with PBS twice, supplemented with 10 μmol/L rhodamine B (reference group with 0.5% DMSO) for 48 h, then washed.

The total RNA was extracted with Trizol according to the kit’s instruction. The purity of the total RNA was determined by UV in OD260/OD280 (>1.8) and the dimer was used as an internal standard to normalize protein levels.

Western blot was performed to analyze cytochrome C levels

Cells at a density of 2×10^5/well were seeded into 6-well plates and incubated at 37 °C for 24 h. Curcumin of 0, 5, 10, 20 and 40 μmol/L was added to the wells and co-incubated for 24 h. And then the medium was removed and total protein was extracted from A549 cells with RIPA lysis buffer. Western blot was performed to analyze cytochrome C levels.

Statistical analysis

Data are expressed as mean ± SEM. The statistical significance of differences between means was determined by one-way analysis of variance (one-way ANOVA) followed by Dunnett’s test or Newman-Keuls post hoc test (SPSS version 13.0 software). A value of P<0.05 was considered to be statistically significant.

Results

Effects of curcumin on A549 cell proliferation

To evaluate the anti-proliferation effect of curcumin on A549 cells in vitro, CCK-8 was used as described above. The results showed that curcumin could inhibit cells proliferation in a dose independent manner. We found that in the same time interval, the rates mounted with the concentration of curcumin increasing, and were statistical different from the control group significantly (P<0.05).
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Figure 2. RT-PCR to determine the effect of curcumin on the expression of Bcl-2 and Bax in A549 cells after treated with 0-40 μmol/ml of curcumin for 24 h. Changes in A549 cells were expressed as the ration compared to GAPDH. Curcumin down regulated the level of Bcl-2 but up-regulated the level of Bax in A549 cells.

Figure 3. RT-PCR to Determine the Effect of Curcumin on the Expression of Bcl-2 and Bax in A549 Cells after Treated with 0-40 μmol/ml of Curcumin for 24 h. The ratio of Bcl-2/Bax was decreased with the dose increased.

Figure 4. Curcumin Can Significantly Lower Mitochondrial Membrane Potential (P < 0.05).

Figure 5. Western Blot was Used to Detect Cytochrome C Release

Discussion

Curcumin, the bioactive component extracted from turmeric rhizome Curcuma longa Linn, has been widely studied with respect to its potential anti-cancer activity in vivo and in vitro. The mechanisms involved are complicated. Curcumin can promote inhibition or arrest cell cycle at all stages through increasing p53 and p21 expression (Jaiswal et al., 2002). In addition, curcumin
inhibits cancer cell growth by stimulating the activation of caspase-3, caspase-7 and caspase-8 (Notarbartolo et al., 2005; Howells et al., 2007). Furthermore, curcumin affects cancer cell proliferation by suppressing TNF-induced NF-xB-dependent gene products (COX-2, cyclin-D, c-myc) (Aggarwal et al., 2006).

Bcl-2 family proteins, which have either pro- or anti-apoptotic activities, have been studied intensively for the past decade owing to their importance in the regulation of apoptosis, tumorigenesis and cellular responses to anti-cancer therapy (Youle et al., 2008). In the Bcl-2 protein family, proapoptotic member Bax and antiapoptotic member Bcl-2 are the active effectors and regulators, and the ratio between Bcl-2 and Bax affects apoptosis induction (Pettersson et al., 2002). According to Sato et al, there are two non-mutually exclusive possibilities: (i) Bcl-2 could induce a pathway that actively maintains cell survival, with Bax serving as a negative regulator of Bcl-2, or (ii) Bax could directly or indirectly generate death signals, with Bcl-2 serving in this case as a dominant inhibitor of Bax (Sato et al., 1994).

In the present study, RT-PCR were adopted to analyze the levels of Bcl-2 and Bax in the A549 cells. Statistical analysis revealed that curcumin stimulated the expression of Bax but inhibited the expression of Bcl-2 in the treatment group. We also observed the significant differences of Bax/Bcl-2 between the treatment group and the control group. These data are consistent with curcumin-inhibited cancer cell growth associated with the balance of Bcl-2/Bax.

There are two main ways of cell apoptosis. One way is to activate apoptotic enzyme caspase within the cell through extracellular signals, the other way is the mitochondrial pathway. Mitochondria play an important role in the process of apoptosis. Mitochondrial membrane potential stimulates the mitochondrial membrane to open, resulting in the release of cytochrome C into cytoplasm, activation of the caspase pathway, and degradation of important intracellular proteins, consequently inducing apoptosis.

The results of this study showed that using curcumin in cells for 40 h can significantly reduce the mitochondrial membrane potential in a dose-dependent manner. In addition, Western blot confirmed the release of cytochrome C from mitochondria to the cytoplasm, suggesting that the mitochondrial apoptosis pathway is important in curcumin-induced lung cancer cell line apoptosis.

In conclusion, this paper indicates that curcumin is a potential growth inhibitor of human non-small cell lung cancer A549 cells. And we have demonstrated the possible anti-cancer mechanism may be the modulation of Bcl-2/Bax and affect the mitochondrial apoptosis pathway by curcumin. These findings are consistent with many studies and may provide a molecular basis for the development of novel natural anticancer agents for NSCLC.

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References


