Journal of
Cell Science and Apoptosis

Research Article Open Access

Curcumin Induces Lung Cancer A549 Cells Apoptosis through Endoplasmic Reticulum Stress Pathway

Li-Le Wang[1], Rui-Cheng Hu[1,2]*, Shuang-Xiang Tan[1,2] and Ming Xu[3]

[1]*Department of Respiratory Medicine, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, China*
[2]*Hunan Province Institute of Gerontology, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, China*
[3]*Department of Gastroenterology Medicine, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, China*

Abstract

Aims: The effects of curcumin on proliferation and apoptosis of lung cancer A549 cells were detected, and the role of endoplasmic reticulum stress reaction was further explored.

Methods: Lung cancer A549 cells were treated with curcumin from different concentrations (2.5 μM, 5 μM, 10 μM, 20 μM, 50 μM), MTT assay detected the impact of curcumin on cell proliferation and flow cytometry determined the effect of curcumin on A549 cells apoptosis. Western blotting detected the changing expression of the target gene on protein level.

Results: Compared with the control group, each group of curcumin (2.5 μM, 5 μM, 10 μM, 20 μM, 50 μM) inhibited the proliferation of A549 cells, and the inhibition of proliferation was more significant with the increase of the time ($p<0.05$). Curcumin can induce apoptosis of A549 cells, compared with the control group ($p<0.05$), showing a dose-dependent relationship. In terms of the protein level, curcumin can induce the expression of activating transcription factor 6 (ATF6) ($p<0.05$), caspase-4($p<0.05$) and C/EBP-homologous protein (CHOP) ($p<0.01$) in A549 cells, and its expression is dose dependent.

Conclusion: Curcumin can induce apoptosis in lung cancer A549 cells through endoplasmic reticulum stress pathway.

Keywords: Curcumin; Endoplasmic reticulum stress; A549 cell; Apoptosis

Introduction

Lung cancers are the worldwide leading killer and do prominent harm to human health than other tumors in current. The side effects of chemotherapeutic drugs are obvious, and it is easy to produce drug resistance, and the advanced lung cancer is not sensitive to chemotherapy [1]. Therefore, searching for a highly effective and low toxic chemotherapy drug for lung cancer has become an urgent problem for many researchers. Since Kuo and others first proposed the possible anti-tumor mechanism of curcumin [2], a large number of experimental studies have confirmed the anti-tumor effect of curcumin. It is suggested that curcumin has inhibitory effects on nasopharyngeal carcinoma, ovarian cancer, colon cancer, breast cancer, lung cancer and so on [3-9]. Researchers found that curcumin can play an anti-cancer role by inhibiting tumor cell proliferation and inducing tumor cell apoptosis [10,11]. However, few studies have reported that curcumin can play a role in lung cancer through which signal pathway. This study used A549 cells to study the effects of curcumin on the proliferation and apoptosis of lung cancer cells, and to elucidate the relevant mechanisms, providing a theoretical basis for the clinical treatment of lung cancer [12-20].

Materials and Methods

Experimental materials

A549 cells come from Central Laboratory; Hunan Normal University, China, Curcumin is from Sigma Aldrich, America [21-24]. Tryptan-EDTA, Fetal bovine serum (FBS) and Triton X-100 are all from Gibco, America. PBS buffer solution, RPMI-1640 comes from Hyclone, America. MTT solution is from Kai Ji biology Ltd. CO, Jiangsu, China. Annexin V and Propidium Iodide (PI) are all from Invitrogen, America. The antibodies including caspase-4, C/EBP-homologous protein (CHOP) and activating transcription factor 6 (ATF6) all come from Santa Cruz, America.

Cell culture

A549 cells were inoculated in the DMEM cell culture medium containing 10% fetal bovine serum, 100 U. ml^{-1} penicillin and 100 U.ml^{-1} streptomycin, and were cultured at 37°C, saturated humidity and the concentration of CO_2 is 5%. The cell culture medium was washed and replaced every other day with the sterilized PBS. When the cell density was more than 85%, the adherent cell were digested with trypsin, and the passages and frozen cells were carried out according to the ratio of 1 to 3. A549 cells with logarithmic growth phase were inoculated and further tested.

MTT assay detected cell proliferation

A logarithmic growth cell trypsin was digested and centrifugated, 200 µl per pore, and the final concentration was 5000 cells per pore, inoculated to 96 orifice plates. Then we respectively use curcumin in different concentrations such as 0 µM, 2.5 µM, 5 µM, 10 µM, 20 µM, 50 µM to process the cells for corresponding 12-hr, 24-hr, 48-hr. Next,

***Corresponding author:** Rui-Cheng Hu, Department of Respiratory Medicine, Hunan Provincial People's Hospital/The First Affiliated Hospital of Hunan Normal University, Changsha, Hunan Province, China, Tel: +86-731-84762679; Fax: +86-731-84762680; E-mail: huruicheng@hotmail.com

Received December 26, 2018; **Accepted** January 10, 2019; **Published** January 20, 2019

Citation: Wang LL, Hu RC, Tan SX, Xu M (2019) Curcumin Induces Lung Cancer A549 Cells Apoptosis through Endoplasmic Reticulum Stress Pathway. J Cell Sci Apo 2: 111.

Copyright: © 2019 Wang LL, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

we add MTT solution (20 μL) to each hole. Finally, after the plate was placed in the incubator for 3 hours, the absorption value at 490 nm was measured with a plate reader.

Flow cytometry assay detected cells apoptosis

We respectively used curcumin in different concentrations (0 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, 50 μM) to process the cells for 24-hr. The PBS scrubbing cells were pre cooled and the cells were digested without EDTA trypsin. And the cells were dyed by Annexin V and Propidium Iodide (PI) for 30 minutes. Finally, flow cytometry analysis was performed using a fluorescence activated cell sorter.

Western blotting assay detected expression of protein. The expression of protein (ATF6, CHOP, caspase-4) were detected by Western blotting assay. The logarithmic growth cells were inoculated on the six hole plates with 1×10^5 cells per pore. Each hole was added with corresponding concentration of curcumin solution (0 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, and 50 μM) in serum containing medium. After the cells were lysed, we collected the supernatant and then extract the cytosolic protein or nuclear protein in a known manner [7,23]. The protein centrifugation liquid was transferred to the PVDF membrane after SDS-PAGE vertical electrophoresis. We performed equivalent electrophoresis analysis of protein lysates.

Real-time quantitative PCR assay detected expression of mRNA

The expression of mRNA of target gene CHOP was detected by real-time quantitative PCR assay. Total RNA was isolated from cells using an RNA queous kit after treatment with curcumin. The high capacity cDNA library kit was used to obtain the first strand cDNA of mRNA. The mRNA levels of CHOP, ATF6 and caspase-4 were quantified by special gene expression kits. Polychromatic real-time polymerase chain reaction (PCR) detection system (Eppendorf, Hamburg, Germany) was used to detect the mRNA of target and control genes. PCR Primer sequence: CHOP, sense strand, 5'-ATGAATCTGCACCAAGCATGA-3', antisense strand, 5'-CAGGTGGGTAGTGTGGCCC-3'; caspase-4, sense strand, 5'-GGGAGAAGGACTTCATTG-3', antisense strand, 5'-TAAGCATGTGATGAGTTG-3'; ATF6, sense strand, 5'-AGCTCCATG CTTAAGGAC-3', antisense strand, 5'-GGGATAGGTGATGATGAA-3' [7,22].

Statistical Analysis

The experimental data were all expressed by average value ± standard deviation (x ± s). The experimental data of curcumin inhibiting A549 cells proliferation were analyzed by variance analysis. As for the comparison between the different concentrations (0 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, 50 μM) of the curcumin treatment group, the Newman-Kells test method was adopted.

Results

Curcumin inhibits proliferation of A549 cells. MTT results showed that curcumin had a significant inhibitory effect on the proliferation of lung cancer A549 cells, and with the prolongation of time and the increase of drug concentration, the inhibitory effect of curcumin was more significant ($p < 0.01$). It showed that curcumin had dose dependence on the proliferation and inhibition of A549 cells (Figures 1 and 2). The figures were obtained by microscope with 200 × amplification. Cells were treated with curcumin (2.5 μM, 5 μM, 20 μM, 50 μM) for 24 hr. a: 2.5 μM; b: 5 μM; c: 20 μM; d: 50 μM. The effects of curcumin on cell relative survival rate in A549 cells. Cell relative survival rate (%) = experimental group OD value/control group OD value ×100%, curcumin (0 μM) which was as a control group. Statistically significant, *P<0.05, #P<0.05, ▲P<0.01, *vs.* vehicle control.

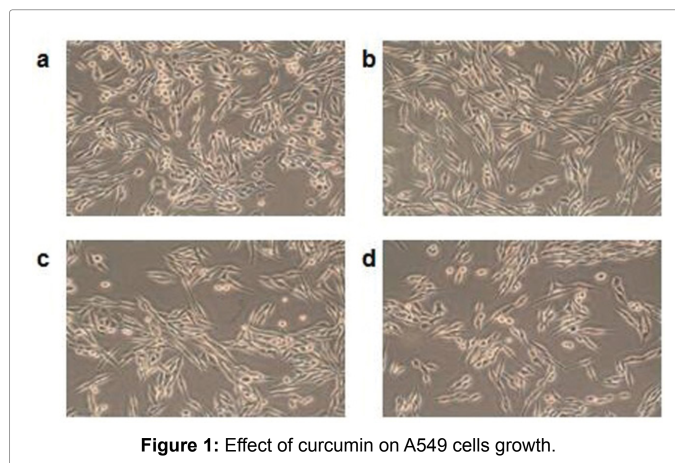


Figure 1: Effect of curcumin on A549 cells growth.

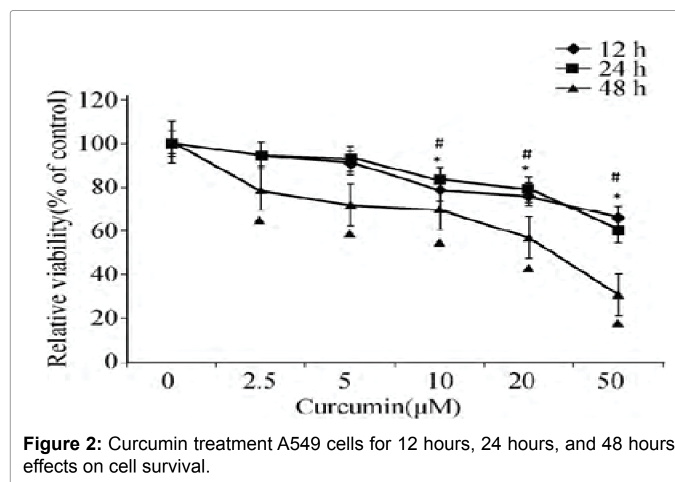


Figure 2: Curcumin treatment A549 cells for 12 hours, 24 hours, and 48 hours effects on cell survival.

Curcumin induces A549 cells apoptosis. After curcumin treatment, apoptotic cells in each group were detected by flow cytometry. The results showed that the apoptosis rate increased with the increase of curcumin concentration. When the concentration of curcumin was 20 μM, compared with the control group, it was statistically significant ($p<0.05$). When the concentration of curcumin was 50 μM, the apoptosis rate was the highest ($p<0.001$) (Figures 3 and 4). Effect of curcumin on the induction of apoptosis in A549 cells as determined by fiow cytometry. A549 cells were treated with curcumin at indicated concentrations for 24 hr, and then stained with annexin V and propidium iodide (PI), followed by detection using fiow cytometry. The cells with apoptosis are shown statistically significant, *p<0.05, ***p<0.001, *vs.* vehicle control.

Curcumin activate the endoplasmic reticulum stress signaling pathway during cells apoptosis. The effect of curcumin was on the expression of ATF6 in A549 cells at the protein level. After the A549 cells were treated with different concentrations of the curcumin (0 μM, 2.5 μM, 5 μM, 10 μM, 20 μM, 50 μM) for 12 hr, the expression of ATF6 increased with the increase of drug concentration. When the concentration of curcumin was 20 μM, the expression of ATF6 was statistically significant compared with that of the control group (0 μM) ($p<0.05$). When the concentration of curcumin was 50 μM, the

expression of ATF6 was the highest (p<0.01) (Figure 5). The effect of curcumin was on the expression of caspase-4 in A549 cells at the protein level.

When the A549 cells were treated after 12 hr with different concentrations of curcumin, the expression of caspase-4 was statistically significant compared with that of the control group (p<0.05) when the concentration of curcumin was 20 μM (p<0.05). When the concentration was 50 μM, the expression of caspase-4 was the highest (p<0.01) (Figure 6). The effect of curcumin was on the expression of CHOP in A549 cells at the protein level. After the A549 cells were treated with curcumin for 12 hr, the expression of CHOP was statistically significant compared with the control group (p<0.05) when the concentration of curcumin was 10 μM (p<0.05), and the expression of CHOP increased obviously with the increase of curcumin concentration (Figure 7). In terms of the RNA level, after the A549 cells were treated with curcumin for 12 hr, expression of CHOP had increased obviously in each group (5 μM, 10 μM, 20 μM, 50 μM) (p<0.01); the expression of ATF6 (20 μM, 50 μM) and caspase-4 (10 μM, 20 μM, 50 μM) had increased slightly (p<0.05) (Figures 7-12). Effect of curcumin on endoplasmic reticulum stress pathway activation. A549 cells were incubated with curcumin at indicated concentrations for 24 hr. The protein of ATF6 was examined

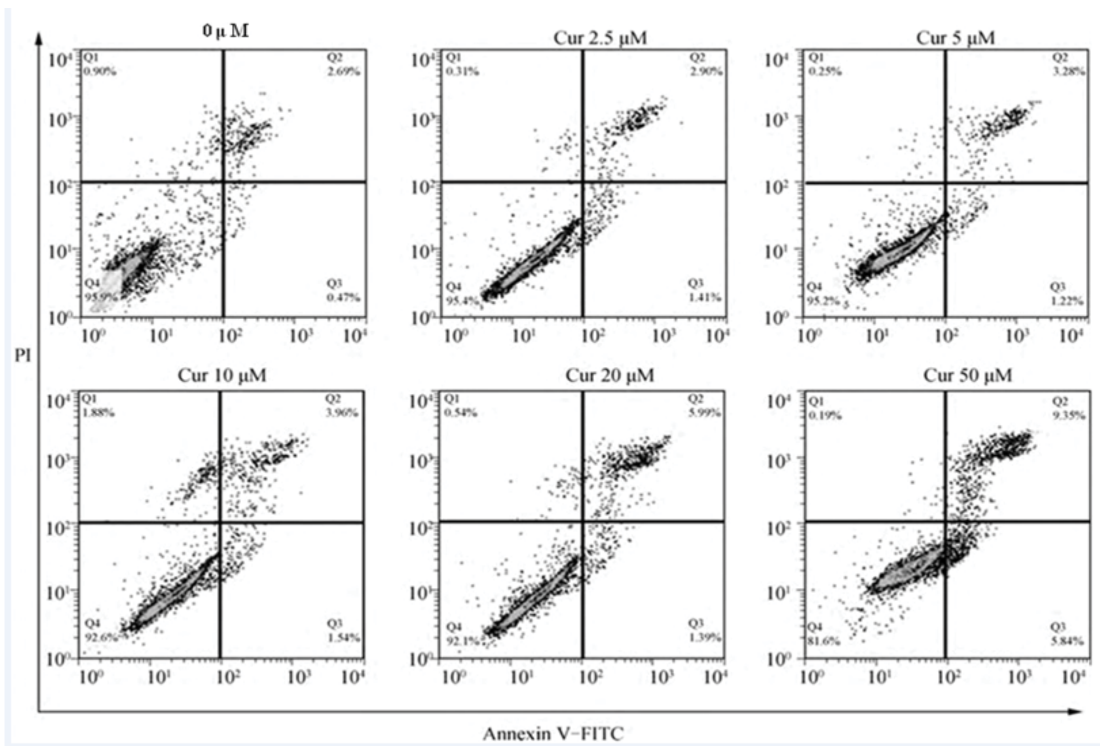


Figure 3: Effect of curcumin on the induction of apoptosis in A549 cells as determined by flow cytometry.

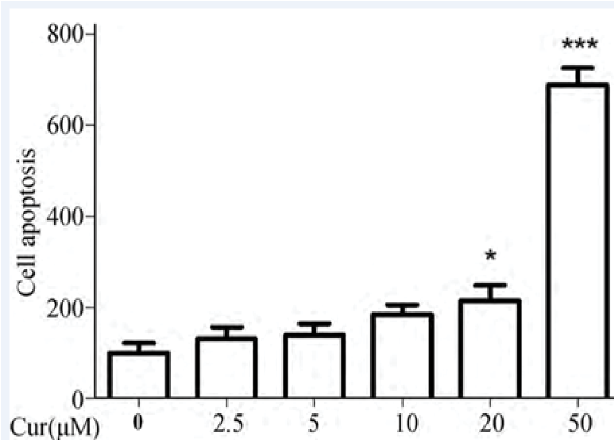
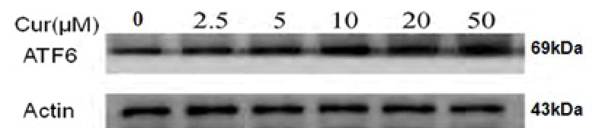


Figure 4: The cells with apoptosis are shown.



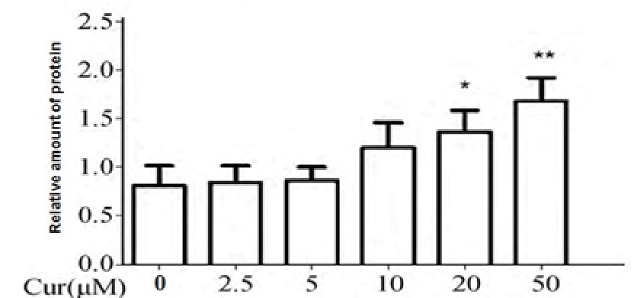


Figure 5: Effect of curcumin on endoplasmic reticulum stress pathway activation.

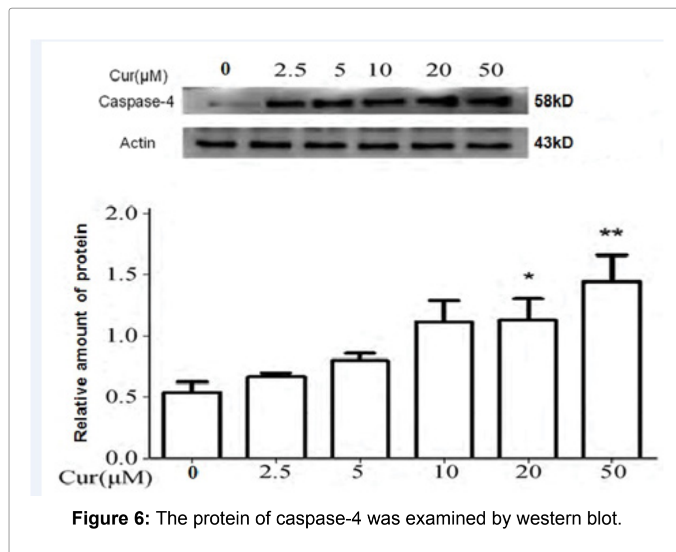


Figure 6: The protein of caspase-4 was examined by western blot.

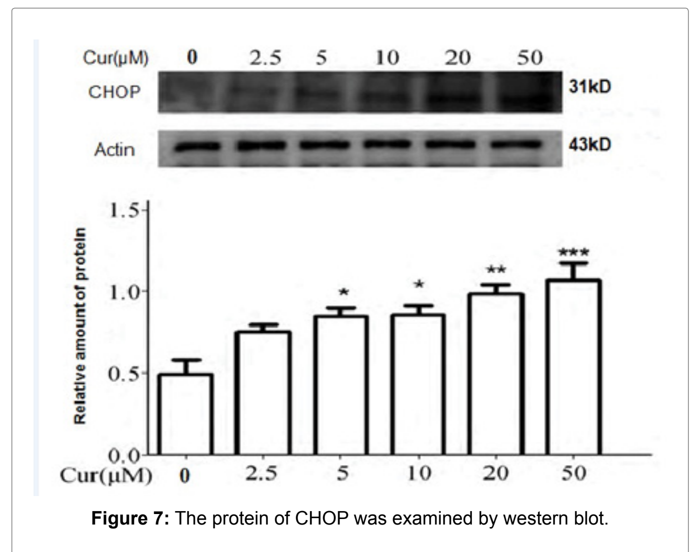


Figure 7: The protein of CHOP was examined by western blot.

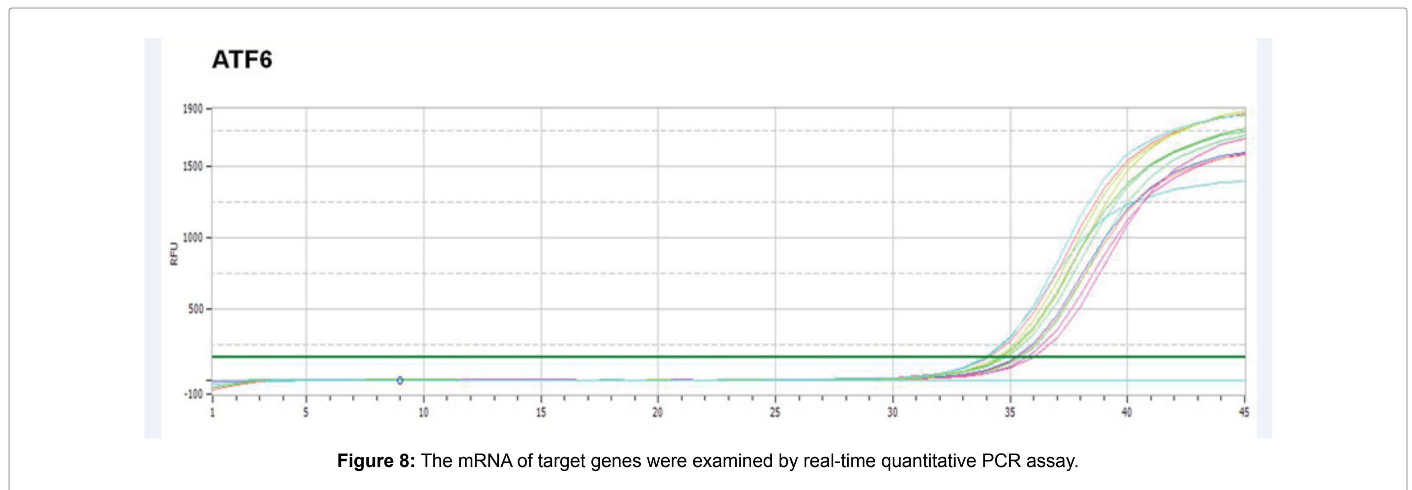


Figure 8: The mRNA of target genes were examined by real-time quantitative PCR assay.

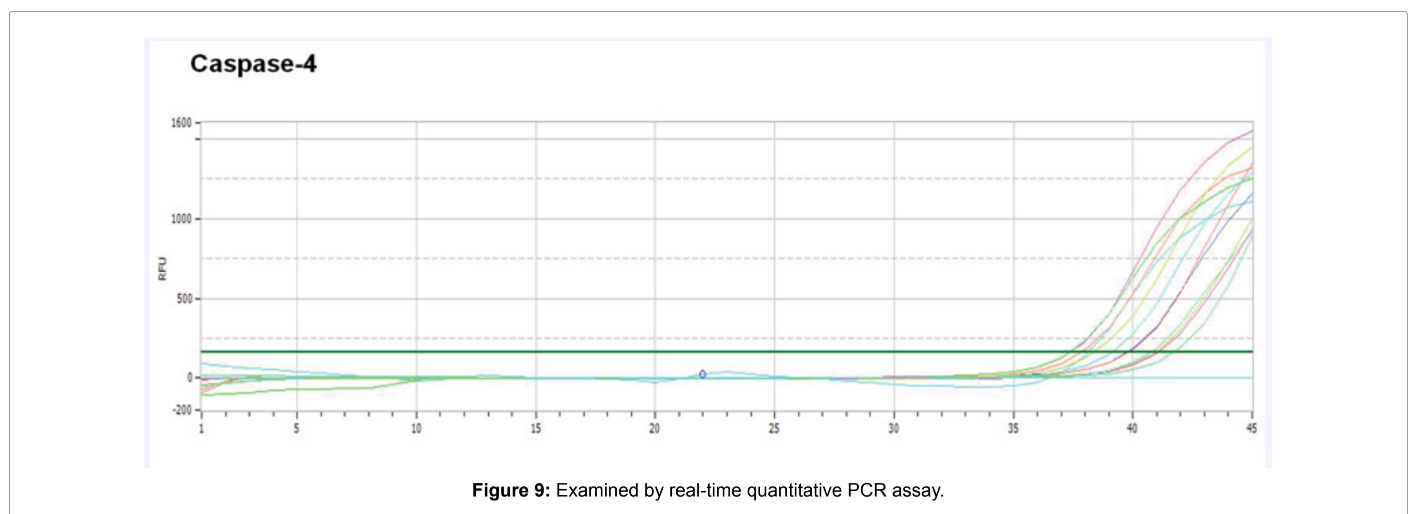


Figure 9: Examined by real-time quantitative PCR assay.

by Western blot. Actin was used as a control for equal loading (*p<0.05, * *p<0.01, *vs.* vehicle group). The protein of caspase-4 was examined by Western blot. Actin was used as a control for equal loading (*p<0.05, * *p<0.01, *vs.* vehicle group). The protein of CHOP was examined by Western blot. Actin was used as a control for equal loading (*p<0.05, **p<0.01, ***p<0.001, *vs.* vehicle group). The mRNA of target genes was examined by real-time quantitative PCR assay. All results were calculated and represented as the percent of vehicle control, actin

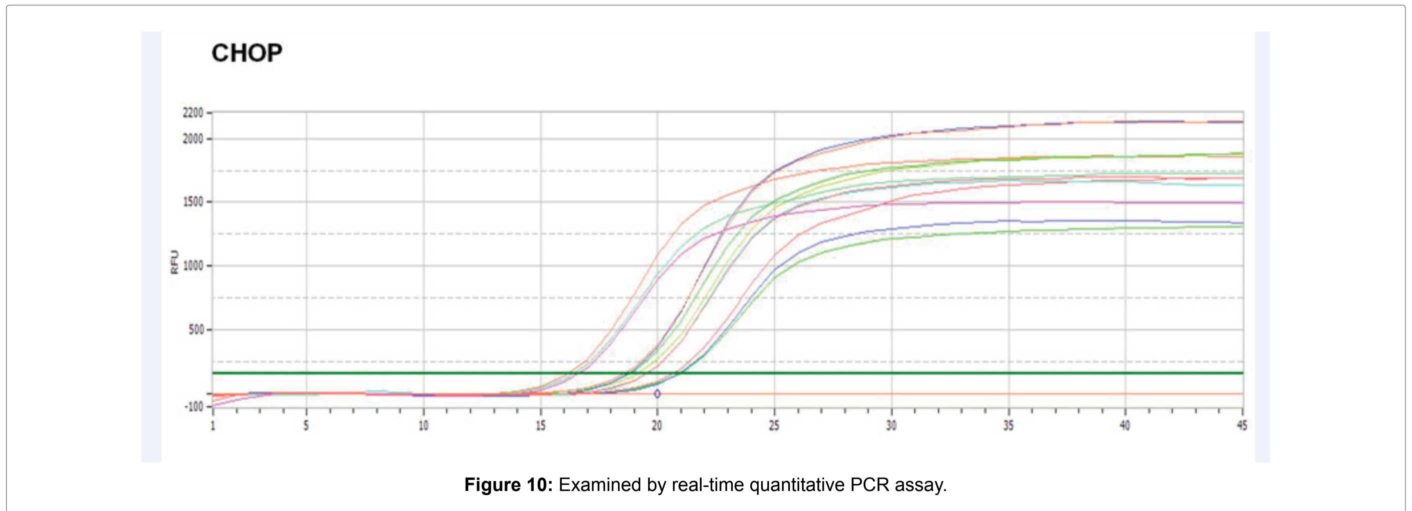


Figure 10: Examined by real-time quantitative PCR assay.

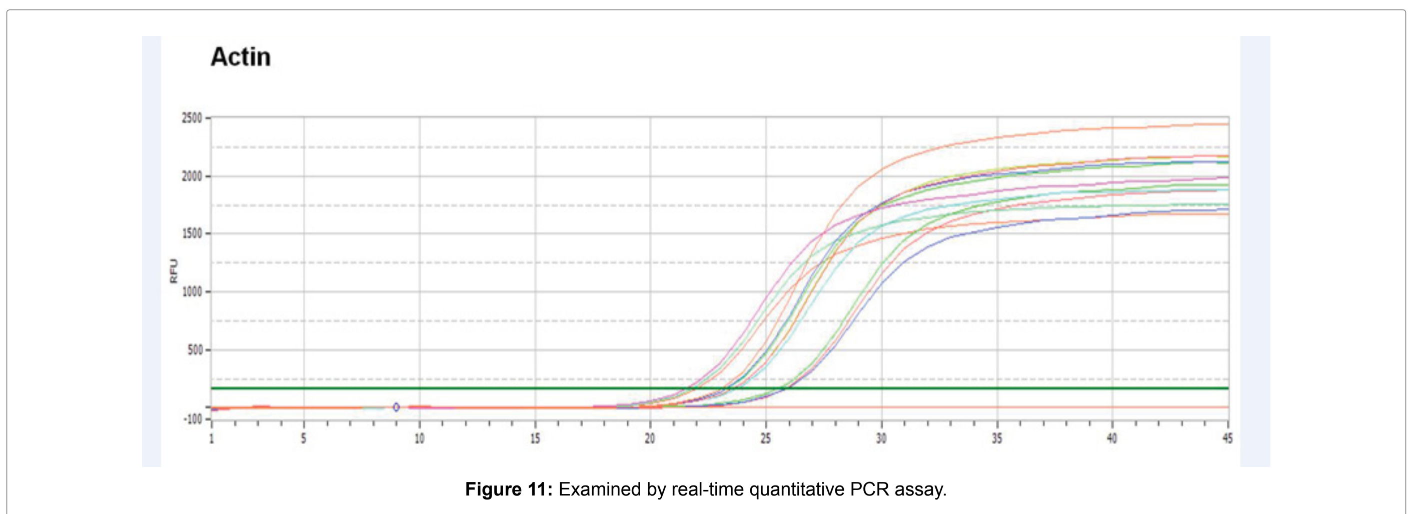


Figure 11: Examined by real-time quantitative PCR assay.

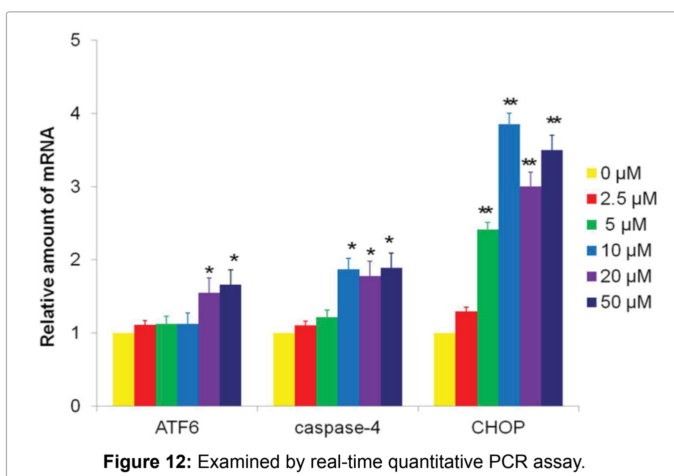


Figure 12: Examined by real-time quantitative PCR assay.

was used as a control for equal loading (*p<0.05, * *p<0.01, *vs.* vehicle group).

Discussion

Lung cancer is a malignant tumor with high malignancy, rapid progress and poor prognosis. Its morbidity and mortality rank first in the world [1]. Therefore, the search for low-toxic and high-efficiency lung cancer chemotherapy drugs has become a hot spot for the treatment of advanced lung cancer. In recent years, Chinese herbal medicine has been further applied in the treatment of tumors, which plays an important role in delaying the progression of the disease and improving the quality of life of patients. Curcumin, as a traditional Chinese herbal medicine, is a polyphenol compound extracted from the rhizomes of the ginger plant [2]. It has anti-oxidation, anti-inflammatory, anti-atherosclerotic and anti-mutagenic effects. Curcumin not only has the pharmacological activity of traditional Chinese medicine, but also has a unique anti-tumor effect.

Conclusion

The results have shown that curcumin has obvious inhibitory effects on a variety of tumor cells, such as lung cancer, breast cancer, cervical cancer, liver cancer and other cancer cells have proliferation inhibition [3-7].

It is found that the anti-tumor effect of curcumin may be induced by inducing apoptosis of tumor cells, and its mechanism of action on each tumor cell is different. The studies have shown that strong and persistent endoplasmic reticulum stress response (ERS) can induce

apoptosis, which is called endoplasmic reticulum stress induced apoptosis (ERSIA) [8-12]. Endoplasmic reticulum (ER) is a protein processing factory of cells, which is mainly responsible for the synthesis and folding processing of membrane proteins and secreted proteins. Many adverse stimuli, such as oxidative stress, hypoxia, cytotoxic substances and nutritional deficiency, can cause the dysfunction of endoplasmic reticulum, resulting in the increased accumulation of wrong folded protein and unfolded protein in endoplasmic reticulum, causing endoplasmic reticulum stress (ERS). If the endoplasmic reticulum stress is too strong or the duration is too long, the cell will initiate an apoptotic program. This apoptosis initiated by endoplasmic reticulum stress is called endoplasmic reticulum stress induced apoptosis (ERSIA).

ERS can induce apoptosis through multiple signaling pathways. Activating transcription factor 6 (ATF6) is a specific chaperone protein on the endoplasmic reticulum, which is closely related to protein folding and modification. An increase in the expression of ATF6 indicates the occurrence of ERS, which is a marker protein for ERS [13-18,24]. Caspase-4 is located in endoplasmic reticulum and is the central link of ERSIA [11,19,20]. C/EBP-homologous protein (CHOP) is a cell stress related factor that plays an important role in cell proliferation and differentiation [12,21-24]. CHOP is hardly expressed under normal physiological conditions of cells, but is highly expressed in endoplasmic reticulum stress, and high expression of CHOP is involved in inducing apoptosis. We found that compared with the control group, the above target gene (ATF6, CHOP, caspase-4) expression increased, which proves that ERS signal pathway is involved in the experimental process. Our experiments suggest that curcumin can induce A549 cells apoptosis through the mechanism of ERS. Although we have confirmed that curcumin can induce A549 cells apoptosis through the ESR signaling pathway, the involvement of other signaling pathways in the process of A549 cells apoptosis remains to be further studied.

References

1. Wong MCS, Lao XQ, Ho KF, Goggins WB, Tse SLA (2017) Incidence and mortality of lung cancer: global trends and association with socioeconomic status. Sci Rep 7: 14300.

2. Kuo ML, Huang TS, Lin JK (1996) Curcumin, antioxidant and antitumor promoter, induces apoptosis in human leukemia cells. Biochim Biophys Acta 1317: 95-100.

3. Yan J, Wang Y, Zhang X, Liu S, Tian C, et al. (2016) Targeted nanomedicine for prostate cancer therapy: docetaxel and curcumin co-encapsulated lipid-polymer hybrid nanoparticles for the enhanced anti-tumor activity in vitro and in vivo. Drug Deliv 23: 1757-1762.

4. Wang JL, Ma WZ, Tu PF (2015) Synergistically Improved Anti-tumor Efficacy by Co-delivery Doxorubicin and Curcumin Polymeric Micelles. Macromol Biosci 15: 1252-1261.

5. Wang XP, Wang QX, Lin HP, Chang N (2017) Anti-tumor bioactivities of curcumin on mice loaded with gastric carcinoma. Food Funct 8: 3319-3326.

6. Luo B, Lee AS (2013) The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene 32: 805-818.

7. Sun Y, Abdul Aziz A, Bowles K, Rushworth S (2018) High NRF2 expression controls endoplasmic reticulum stress induced apoptosis in multiple myeloma. Cancer Lett 412: 37-45.

8. Sano R, Reed JC (2013) ER stress-induced cell death mechanisms. Biochim Biophys Acta 1833: 3460-3470.

9. Zhao Y, Zhu C, Li X, Zhang Z, Yuan Y (2011) Asterosaponin 1 induces endoplasmic reticulum stress-associated apoptosis in A549 human lung cancer cells. Oncol Rep 26: 919-924.

10. Chen J, Wang FL, Chen WD (2014) Modulation of apoptosis-related cell signalling pathways by curcumin as a strategy to inhibit tumor progression. Molecular Biology Reports 7: 4583-4594.

11. Xiao J, Wang Y, Peng J, Guo L, Hu J, et al. (2012) A synthetic compound, 1,5-bis (2-methoxyphenyl) penta-1,4-dien-3-one (B63), induces apoptosis and activates endoplasmic reticulum stress in non-small cell lung cancer cells. Int J Cancer 131: 1455-1465.

12. Zhu GY, Wong BC, Lu A, Bian ZX, Zhang G, et al. (2012) Alkyl phenols from the roots of ardisia brevicaulis induce g1 arrest and apoptosis through endoplasmic reticulum stress pathway in human non-small-cell lung cancer cells. Chem Pharm Bull 60: 1029-1036.

13. Wei J, Rahman S, Ayaub EA, Dickhout JG, Ask K (2013) Protein misfolding and endoplasmic reticulum stress in chronic lung disease. Chest 143: 1099-1105.

14. Park S, Lim W, Bazer FW, Song G (2017) Naringenin induces mitochondria-mediated apoptosis and endoplasmic reticulum stress by regulating MAPK and AKT signal transduction pathways in endometriosis cells. Mol Hum Reprod 23: 842-854.

15. Zhou B, Tan J, Zhang C, Wu Y (2018) Neuroprotective effect of polysaccharides from Gastrodia elata blume against corticosterone induced apoptosis in PC12 cells via inhibition of the endoplasmic reticulum stress mediated pathway. Mol Med Rep 17: 1182-1190.

16. Seo SU, Min KJ, Woo SM, Kwon TK (2018) Z-FL-COCHO, a cathepsin S inhibitor, enhances oxaliplatin-mediated apoptosis through the induction of endoplasmic reticulum stress. Exp Mol Med 50: 107.

17. Lam M, Lawrence DA, Ashkenazi A, Walter P (2018) Confirming a critical role for death receptor 5 and caspase-8 in apoptosis induction by endoplasmic reticulum stress. Cell Death Differ 25: 1530-1531.

18. Woo M, Noh JS, Cho EJ, Song YO (2018) Bioactive Compounds of Kimchi Inhibit Apoptosis by Attenuating Endoplasmic Reticulum Stress in the Brain of Amyloid β-Injected Mice. J Agric Food Chem 66: 4883-4890.

19. Lee AS (2007) ATF6 induction in cancer: therapeutic and prognostic implications. Cancer Res 67: 3496-3499.

20. Li T, Su L, Zhong N, Hao X, Zhong D, et al. (2013) Salinomycin induces cell death with autophagy through activation of endoplasmic reticulum stress in human cancer cells. Autophagy 9: 1057-1068.

21. Choi AY, Choi JH, Yoon H, Hwang KY, Noh MH, et al. (2011) Luteolin induces apoptosis through endoplasmic reticulum stress and mitochondrial dysfunction in Neuro-2a mouse neuroblastoma cells. Eur J Pharmacol 668: 115-126.

22. Moriya S, Miyazawa K, Kawaguchi T, Che XF, Tomoda A (2011) Involvement of endoplasmic reticulum stress-mediated CHOP (GADD153) induction in the cytotoxicity of 2-aminophenoxazine-3-one in cancer cells. Int J Oncol 39: 981-988.

23. Gan GX, Hu RC, Dai AG, Tan SX, Ouyang Q, et al. (2011) The role of endoplasmic reticulum stress in emphysema results from cigarette smoke exposure. Cell Physiol Biochem 28: 725-732.

24. Liu CM, Zheng GH, Ming QL, Sun JM, Cheng C (2013) Protective effect of quercetin on lead-induced oxidative stress and endoplasmic reticulum stress in rat liver via the IRE1/JNK and PI3K/Akt pathway. Free Radic Res 47: 192-201.