Trends in Immunotherapy (2021) Volume 5 Issue 2.1: Special Issue - Research Progress in Immunotherapy in China
doi:10.24294/ti.v5.i2.1.1371

ORIGINAL RESEARCH ARTICLE

Studies on the proliferation inhibition effects of TUA from Actinidia chinensis Radix on lung cancer xenografts in nude mice and its preliminary mechanism

Xiaohua Guo, Haibo Hu, Qi Jin, Hongliang Li, Qilai Cheng*

School of Pharmacy, Gannan Medical University, Ganzhou 341000, Jiangxi Province, China. E-mail: cql_57@126.com

ABSTRACT

Objective: To investigate inhibitory effect of TUA (2β, 3β, 23-trihydroxy-urs-12-en-28-oic acid) isolated from Actinidia chinensis Radix on the lung cancer xenografts in nude mice and explore its preliminary mechanism. **Methods:** NCI-H460 cells were implanted into nude mice and the transplantation tumor block from nude mice of more than 2 generations was inoculated to the right armpits of BALB/c mice with dissecting needle to establish a lung cancer xenograft model. When the transplanted volume was about 50 mm^3, the mice were randomly divided into 6 groups: (1) model group; (2) 10 mg kg^{-1} cisplatin group; (3) 10 mg kg^{-1} PDTC group; (4) TUA high dose group (30 mg kg^{-1}); (5) TUA middle dose group (12 mg kg^{-1}); (6) TUA low dose group (6 mg kg^{-1}). Administration approach was intratumoral injection. The effects of each group on the weight of transplanted tumor animals, the volume and weight of tumor were continuously observed for 14 days. Tumor volume growth curve was drawn and tumor inhibitory rate and index were calculated; HE staining was used to observe nude mice tumor tissue pathological changes. The effects of TUA on NF-κB signaling pathway related proteins were detected by immunohistochemistry and Western blot. **Results:** *In vivo* experiments showed that the transplanted tumors in nude mice became smaller compared with the models. With the increase of TUA dose, the tumor tissue became smaller and smaller, especially in high TUA dose (30 mg kg^{-1}). It had the similar size with the NF-κB inhibitor PDTC (10 mg·kg^{-1}) group. HE dyeing observation results confirmed the degree of tumor necrosis and fission in TUA treated tumor tissues obviously decreased. Immunohistochemical results showed that comparing the TUA treatment group with the model group, p65 expression in tumor tissues was reduced, and expression of IκBα increased. Western blot results also showed that the NF-κB related p65 protein expression levels decreased, at the same time IκBα protein expression level increased; the apoptosis related proteins Survivin protein expression was depressed, Caspase-3 protein expression was promoted. **Conclusion:** TUA significantly inhibits the growth of lung transplantation tumor and its mechanism. It may be related to the decreasing the expression of p65, Survivin and increasing the expression of IκBα, Caspase-3 in tumor tissues.

Keywords: 2, 3β, 23-Trihydroxy-urs-12-en-28-oic Acid; Lung Cancer; Xenograft; Apoptosis-Related Proteins

ARTICLE INFO

Received 29 August 2021
Accepted 24 October 2021
Available online 29 October 2021

1. Introduction

At present, lung cancer is still the main cause of mortality of cancer patients all over the world, among which patients with non-small cell lung cancer (NSCLC) account for almost 85%–90%[1]. The main reasons for the high mortality of lung cancer are the difficulty of early diagnosis, easier metastasis to surrounding tissues and organs, and lower and lower sensitivity to chemotherapeutic drugs[2]. Although the double platinum chemotherapy regimen containing cisplatin and carboplatin has been used for many years in the treatment of more than 50% of NSCLC patients, the 5-year survival rate is only 15.7%–17.5%, and most patients eventually deteriorate and die in the next decade. Alth-

ough many countries in the world have been trying to apply some new strategies for the prevention and treatment of lung cancer, the 5-year survival rate will not exceed 18%[3]. Therefore, in order to improve the survival rate of lung cancer patients, the most urgent task is to develop new clinical drugs, especially some natural products derived from animals and plants, which have extensive biological activities and almost no adverse reactions.

Over the past century, natural products have provided a large number of drug sources for the treatment of human diseases[4]. In recent years, *in vivo* and *in vitro* studies have shown that a considerable number of terpenoids isolated and identified from plants have significant antitumor activities against different kinds of tumor cell lines. Some of them have been successfully applied to the prevention of clinical human tumor diseases, especially some pentacyclic triterpenoids compounds such as oleanolic acid, glycyrrhetic acid and carbenoxolone[5]. Therefore, the research and development of new pentacyclic triterpenoids and their derivatives with stronger antitumor activity have attracted more and more interest of researchers with life science related professional backgrounds such as medicine, biochemistry and molecular biology.

TUA (2β, 3β, 23-trihydroxy-urs-12-en-28-oic acid) is one of the main active components of Ursane type triterpenoids extracted, isolated and identified from Actinidia chinensis Radix, a traditional Chinese medicine. Actinidia chinensis Radix, also known as Chinese actinidia root, is a folk Chinese herbal medicine widely used in Jiangxi, Guangxi and Sichuan. It has always been said that it can treat "unknown swelling poison" among the people. It has been used in many areas to prevent and treat tumors and has achieved good curative effects, including gastric cancer, esophageal cancer, liver cancer, and lung cancer[6]. A large number of studies have shown that Ursane type triterpenoids and their derivatives with the similar structure to TUA have a wide range of pharmacological activities, such as antioxidant effect, anti-immune effect, neuroprotective effect, and anti-tumor effect[7].

Previous studies of our research group showed that TUA could inhibit the proliferation of lung cancer cell NCI-H460 to induce tumor cell apoptosis *in vitro*[8]. In this experiment, the lung cancer cell NCI-H460 cell line was used to prepare the nude mouse transplantation tumor model and TUA was taken as the research object to observe the effect of TUA application on the NCI-H460 xenografts *in vivo* and explore the mechanism of action, which can provide a theoretical basis for the further elaboration of the potential chemoprevention use of cancer.

2. Materials

2.1 Drugs (reagent)

TUA was isolated from the root of Actinidia chinensis Radix (Actinidiaceae) in this laboratory. The Actinidia chinensis Radix was separated by solvent extraction and various column chromatography. The obtained compounds were identified by physical and chemical analysis and spectral technology, and finally the Ursane triterpenoids were obtained. The purity of TUA was examined by Agilent 1100 HPLC, DAD detector and the area normalization method. The content was more than 97%. In this experiment, TUA was dissolved with dimethyl sulfoxide (DMSO). The final concentration of solvent DMSO shall not exceed 0.1%. The drugs used in the experiment were generally stored in –4 ℃ refrigerator for no more than 4 days. The concentration of drugs was adjusted by RPMI-1640 medium; using it right after it was ready. Roswell Park Memorial Institute 1640 medium (RPMI1640, GIBCO Company, USA); SDS (Bio-Rad company, Batch No.: 210008353); DAB color development kit (Zhongshan Jinqiao, Batch No.: K133319D); various antibodies: (1) GAPDH high-quality internal reference labeled with internal reference antibody HRP (Shanghai Kangcheng Biology, Article No.: KC-5G5); (2) primary antibody name: Caspase-3 (novusibo, Article No.: NB500-210), corresponding secondary antibody: Rabbit Anti-Mouse IgG (H + L)-HRP; primary antibody name: Survivin (Cell signaling, Article No.: 2802), corresponding secondary antibody: Rabbit Anti-Mouse IgG (H + L)-HRP; primary antibody name: p65, IκBα (abcam, Article No.: 16502), corresponding secondary antibody: Goat Anti-Rabbit IgG (H + L), Mouse/Human ads-HRP; (3) secondary antibody

name: Goat Anti-rabbit IgG (H + L), Mouse/Human ads-HRP (southern biotech, Article No.: 4050-05); secondary antibody name: Rabbit Anti-Mouse IgG (H + L)-HRP (southern biotech, Article No.: 6170-05); DMSO and all other biochemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Key instruments

Inverted optical microscope (CKX41, OLYMPUS); cell constant temperature incubator (HERACELL150i, Thermo scientific); desktop high-speed frozen centrifuge (H1650R, Shanghai Luxiangyi); microplate reader (SPECTRA max Plus 384, Molecular Devices, Inc); electrophoresis system (Mini-Proten Tetra System, Bio-RAD Corporation); gel imager: (ChemiDoc XRS + System, Bio-RAD Corporation).

2.3 Cell system and experimental animals

The human lung cancer cell line NCI-H460 was purchased from the Shanghai Cell Bank, Chinese Academy of Sciences. 70 SPF-level BALB/c nude mice, 18–22 g, 6–8 weeks old, female, were purchased from Shanghai Slac Laboratory Animal Co., Ltd. with license No.: SCXK (Shanghai) 2012–0002.

3. Experimental methods

3.1 Cell culture and treatment

NCI-H460 cells were placed in RPMI 1640 medium (which mainly contained 10% fetal bovine serum (FBS), 100U mL^{-1} penicillin, 2 mmol L^{-1} L-glutamine, and 100 mg L^{-1} streptomycin, *etc.*) and routinely cultured in a confined incubator with 95% saturated humidity and 5% CO_2, at 37 ℃. Cell growth was observed daily, with a single passage cycle of 3 days. All subsequent experiments used normally cultured NCI-H460 cells in the log growth phase. Meanwhile, the final concentration of dimethyl sulfoxide (DMSO) of the dissolved samples (test drug and control products) was ≤0.1%.

3.2 Preparation of the transplanted tumor animal model

Collect NCI-H460 cells in logarithmic growth stage, wash them with serum-free and antibiotic-free RPMI-1640 culture medium for 3 times, count the cells by flow cytometry, adjust the cell density, conduct animal experiments in the ultra-clean stage, briefly disinfect them with alcohol cotton. Then blow and mix the cell suspension with 1 mL sterile syringe, and inoculate 0.1 mL subcutaneously on the right side of nude mice for 5×10^5 cells/animal to prepare tumorigenic animals. After 2 weeks, the subcutaneous tumor tissue was taken, soaked in precooled PBS. The tumor capsule and internal tissue were removed, the active tissue was cut into small squares of about 1 cm^3, and the anatomical needle was inserted into the subcutaneous part of the right limb of nude mice.

3.3 Tumor suppressor experiments

Three days after inoculation, the growth of nude mice was observed every day. The action, response to external stimuli, diet, fecal color and weight (W) of nude mice were observed, as well as the tumor growth. After the third day of transplantation, when the transplanted volume was about 50 mm^3, the mice were randomly divided into 6 groups. Intraperitoneal administration was started and the tumor diameter was measured. The specific grouping is as follows: (1) the model group: given normal saline after tumor inoculation; (2) the positive control group 1: given cisplatin after tumor inoculation, with a dose of 10 mg kg^{-1}; (3) the positive control group 2: given NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC), with a dose of 10 mg kg^{-1}; (4) the TUA high dose group: with a dose of 30 mg kg^{-1}; (5) the TUA medium dose group: with a dose of 12 mg kg^{-1}; (6) the TUA low dose group: with a dose of 6 mg kg^{-1}. The longest diameter (a) and the shortest diameter (b) of the tumor were measured with a vernier caliper, and the tumor volume (V) was calculated. $V\ (cm^3) = 1/6\pi ab^2 = 0.5236ab^2$. Draw tumor volume growth curve. At the end of the experiment, the nude mice were killed by cervical dislocation. The subcutaneous tumor blocks were taken, weighed and photographed, soaked in 4% paraformaldehyde for fixation, and half of the tumor samples from each group were frozen in liquid nitrogen (–80 ℃) for subsequent pathological sections, such as HE staining, immunohistochemistry and Western blotting. A. growth index: the longest

diameter (a) and shortest diameter (b) were measured for 4 to 5 times per week, and the calculated volume: $V = 1/6\pi ab^2 = 0.5236ab^2$; then draw the tumor volume growth curve. B. tumor index: tumor index (mg/g) = tumor weight (mg)/mouse weight (g). C. tumor suppressor rate (%) = [(1 – T/C)] × 100%. In the formula, T means the average tumor weight of the test drug group; C means the average tumor weight of the control group (the model group, tumor bearing animals without being given drugs).

3.4 Using HE staining to observe the histopathological changes of tumors in nude mice

3.4.1 Making slices

The tumor tissue was fixed in 4% formaldehyde solution for–5 days. Remove the tissue from the fixed solution and trim it to an appropriate shape and thickness. The tissue blocks were dehydrated by 80%, 90%, 95%, 100% ethanol I, 100% ethanol II and 100% ethanol III, given transparent treatment carried with xylene I for 45 min, and xylene II for 15 min, and soaked in wax for 5 h. According to the principle that the material is taken from the bottom, the tissue is embedded with paraffin, and the wax block is placed at –20 ℃ for refrigeration after cooling and solidification. Make slices (with 4 μm thickness) and put the slices into 65 ℃ incubator for 6–12 h; box and store at room temperature.

3.4.2 HE staining

Xylene I for 15 min, xylene II for 15 min, absolute ethanol I for 5 min, absolute ethanol II for 5 min, 95% ethanol for 5 min, 80% ethanol for 5 min, tap water immersion for 1 min. Immerse the slices in Hematoxylin dyeing solution, dye at room temperature for 5 min, and wash with tap water for 1 min. Immerse the slices in 1% hydrochloric acid alcohol solution for a few seconds and in tap water until the tissue returns to blue. Immerse the slices in Eosin staining solution for 3–5 min, and wash off the floating color on the slide with tap water. 80% ethanol for 0.5 min, 95% ethanol I for 0.5 min, 95% ethanol II for 0.5 min, absolute ethanol I for 0.5 min, absolute ethanol II for 0.5 min, xylene I transparent for 3 min, xylene II transparent for 3 min. After taken out, they were sealed with neutral gum, observed with microscopic and photographed; staining results were analyzed.

3.5 Using immunohistochemistry (IHC) to detect the expression of p65 and IκBα in tumor tissues

3.5.1 Making slices

Tissue slices were placed in a 65 ℃ incubator for 6–12 h.

3.5.2 IHC to detect the expression of p65 and IκBα in tumor tissues

Using Xylene to dewax. Gradient alcohol rehydration: xylene I for 20 min—xylene II for 20 min—100% ethanol for 5 min—100% ethanol II for 5 min—95% ethanol for 5 min—80% ethanol for 5 min—PBS washing for 3 × 3 min. Block and inactivate endogenous peroxidase: incubate with 3% H_2O_2 at 37 ℃ for 10 min, and rinse with PBS for 3 × 3 min. Antigen repair: microwave repair in 0.01 M citric acid buffer (pH 6.0), natural cooling to room temperature, PBS washing for 3 × 3 min, drop the primary antibody and incubate it in the refrigerator at 4 ℃ to overnight (use PBS buffer to replace the primary antibody as negative control), then turn to room temperature equilibrium for 30 minutes, and rinse with PBS for 3 × 5 min. Then add secondary antibody, incubate at 37 ℃ for 15 min, and rinse with PBS for 3 × 5 min. DAB reaction staining, observe the reaction progress under the microscope, and rinse with tap water; hematoxylin re-staining, drying, and sealing.

3.5.3 Data statistics

Under the microscope, three different visual fields were randomly selected for each immunohistochemical section（× 200) to observe and interpret the positive expression intensity and positive rate. The staining results were analyzed by semi-quantitative analysis: scored by staining intensity combined with the percentage of positive cells.

3.6 Western blot to test the expression of p65, IκBα, Caspase-3

Take an appropriate amount of tumor tissues preserved in liquid nitrogen, add 1 mL of cell lysate containing 1% protease inhibitor, homogenize it in a homogenizer, extract the total protein of cells,

quantify it with BCA. Equal amount of total protein (40 μg) was taken for semi-dry transmembrane with a 10% SDA-PAGE, Bio-Rad transmembrane ometer, and 5% skimmed milk was used to seal. Adding the primary antibody and incubating at 4 ℃ for the night, and washing the membrane. Adding the secondary antibody and incubating at room temperature for 1 h, and washing the membrane. Using ECL to show colors, and analyzing the image by Gel-pro Analyzer software. The protein content was expressed by the ratio of the primary antibody/GAPDH. The dilution ratio of the primary antibody p65, IκBα was 1:2,000, while the dilution ratio of the corresponding secondary antibody Goat Anti-Rabbit IgG (H + L), Mouse/Human ads-HRP was 1:20,000; the dilution ratio of the primary antibody Caspase–3 was 1:500, the dilution ratio of the primary antibody Survivin was 1:1,000, while that of the corresponding secondary antibody Rabbit Anti-Mouse IgG (H + L)-HRP was 1:1,000. GAPDH labeled by HRP was taken as an internal reference with a dilution ratio of 1:10,000.

3.7 Statistical analysis

The experiments were repeated at least 3 times. The data were statistically analyzed by SPSS 17.0 software. The measurement data were expressed with $\bar{x} \pm s$, and the counting data were expressed in rate. The comparison between measurement data groups was tested by independent sample *t*, and analysis of variance was used for multi group comparison. $P < 0.05$ means the difference was statistically significant.

4. Results

4.1 Tumor suppressor experiments

Animal tumorigenesis and growth: 59 of 60 nude mice in this experiment had tumorigenesis, with the tumorigenesis rate of 98.30%. After inoculating the tumor block for 3 days, tumor like nodules with a diameter of about 3–4 mm could be seen, and the texture was soft. After that, the neoplastic nodules became harder and the volume increased significantly. The change of subcutaneous transplanted tumor in nude mice after administration is shown in **Figure 1**, the growth curve after administration for 2 weeks (day 1–day 14) is shown in **Figure 2**, and the tumor weight inhibition rate and tumor index are shown in **Table 1**. It can be seen from **Figure 1** that after administration, the tumor tissue in nude mice became smaller; with the increasing dose of TUA, the tumor tissue became smaller and smaller, especially at the high dose of TUA (30 mg kg^{-1}), and the tumor tissue size was similar to that of NF-κB inhibitor PDTC (10 mg kg^{-1}). Similar results can be obtained from the growth curve in **Figure 2**. The tumor tissue volume in the model group gradually increased (from 59.16 mm^3 to 1177.81 mm^3), and the tumor volume in the administration group increased slowly, which was more obvious at the high dose of TUA (30 mg kg^{-1}) (from 59.16 mm^3 to 429.11 mm^3), further indicating that TUA has a certain inhibitory effect on the growth of tumor tissues. Similar conclusions can be drawn from the tumor weight inhibition rate and tumor index in **Table 1**. Comparing high and medium dose of TUA with model groups, the differences were statistically significant ($P < 0.05$ or $P < 0.01$). The treatment effects of cisplatin and the NF-κB inhibitor PDTC, the drugs commonly used clinically for lung cancer, were also statistically significant when compared with model groups ($P < 0.01$) (**Table 2**).

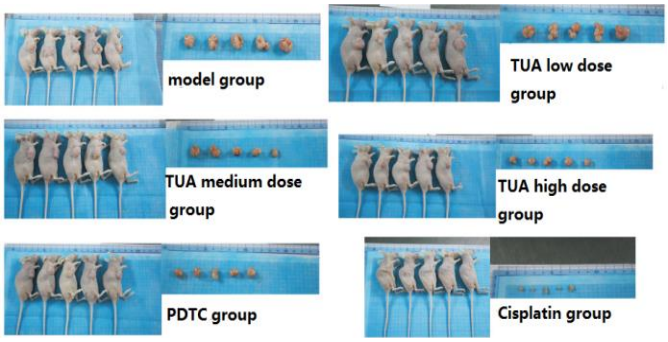


Figure 1. Effect of TUA on the tumor growth in nude mice.

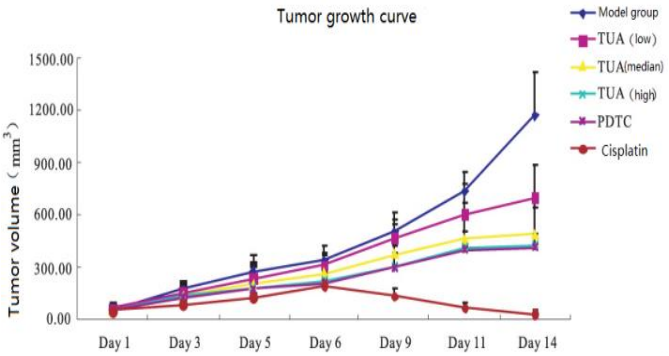


Figure 2. The inhibition effect of TUA on the tumor growth in nude mice (Growth curve).

Table 1. Tumor growth inhibitory rate and tumor markers/$\bar{x} \pm s$, n = 5

Groups	Tumor inhibitory rate /%	*P*	Tumor index	*P*
Model group	0.00 ± 0.00		2.40 ± 12.36	
TUA (high)	85.81 ± 16.33**	0.007	26.52 ± 5.48**	0.006
TUA (median)	58.53 ± 10.54*	0.021	32.07 ± 10.77*	0.042
TUA (low)	45.07 ± 9.95*	0.025	40.62 ± 9.58*	0.018
PDTC	84.23 ± 13.24**	0.003	27.06 ± 6.85**	0.007
Cisplatin	90.31 ± 16.84**	0.008	20.88 ± 5.02**	0.008

Table 2. Analysis of variance results (ANOVA)

Source of difference	Quadratic sum	Free degree	Variance	Value *F*	Value *P*
Between groups	39.45	4	9.86	18.60	0.00
Within group	12.19	23	0.53		
Total difference	51.64	27			

4.2 HE staining

Transplanted tumor tissues by He staining presented blue nucleus, pink cytoplasm and bright red blood cells, which are shown in **Figure 3**. As can be seen from the figure, a large number of tumor necrosis areas and nuclear division phenomenon were visible in the tissue slices of the model group. In the TUA group, similar to the PDTC and cisplatin groups, the degree of tumor necrosis and nuclear division image were significantly reduced, and more collagen fibers can be seen. It is further proved that TUA has some inhibitory effect on the proliferation of transplanted tumors from the perspective of the pathology.

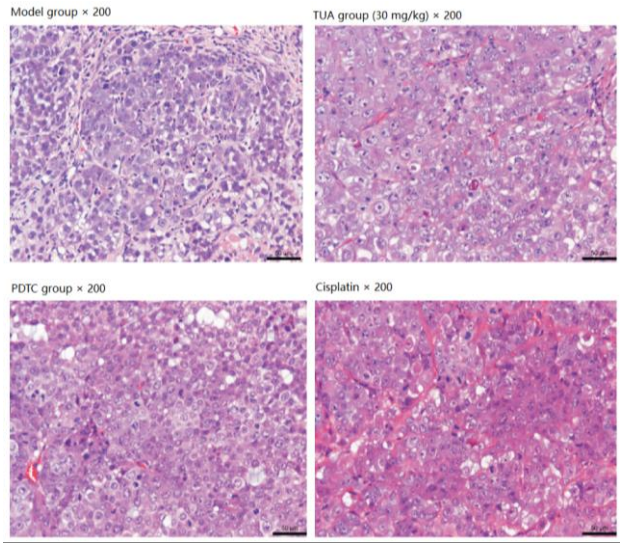
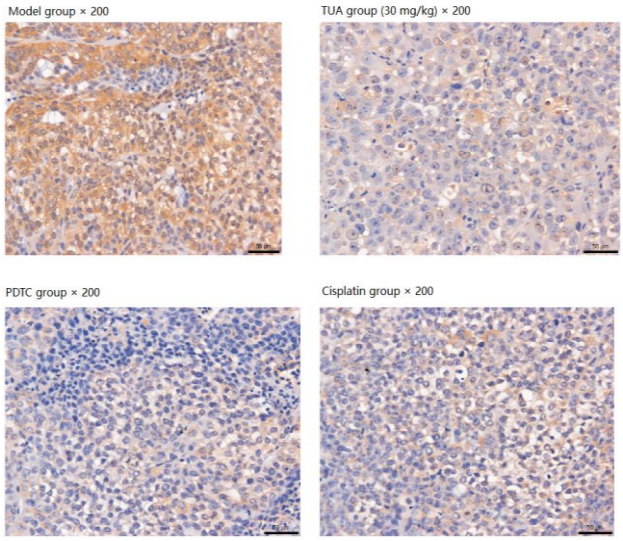


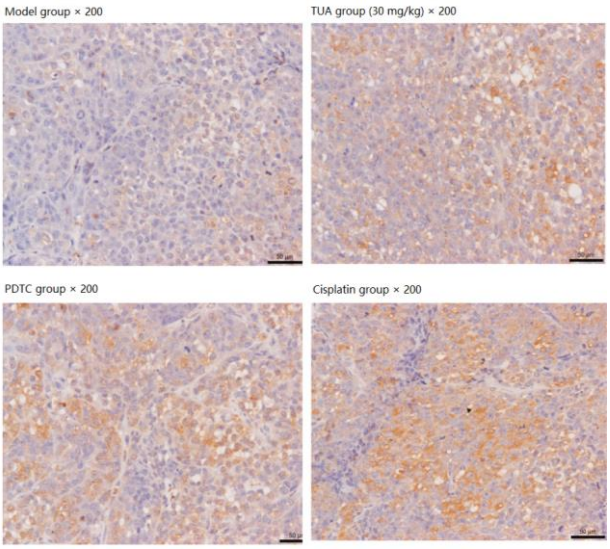
Figure 3. Histopathological changes of tumor after TUA treatment (HE staining).

4.3 Immunohistochemistry

The immunohistochemical method was used to detect the expression of NF-κB (p65), IκBα in transplanted tumor tissues. Immunostaining results are shown in **Figure 4**. It can be seen from the figure that p65 immune positive was mainly located in the nucleus and cytoplasm, while IκBα immunopositivity was mainly localized in the cytoplasm of cells, both of which were brownish yellow. Compared with the model group, the expression of p65 in tumor tissues decreased in the TUA treatment group (**Figure 4A**), while the expression of IκBα increased (**Figure 4B**), and the positive control drugs PDTC and cisplatin also showed similar effects.



A. p65 immunohistochemistry results.



B. IκBα immunohistochemistry results.

Figure 4. Effect of TUA on expressions of p65, IκBα in tumor.

4.4 Western blot results

The changes of apoptosis-related protein ex-

pression in transplanted tumor tissues were analyzed by Western blot, and the results of each treatment group are shown in **Figure 5.** It is known from the figure that the expression level of p65 protein associated with NF-κB decreased, along with the increase of the expression of IκBα protein. Survivin protein expression associated with apoptosis was inhibited and Caspase-3 protein expression was increased. The TUA high-dose group (30 mg kg^{-1}) was comparable to the positive control drugs PDTC and cisplatin.

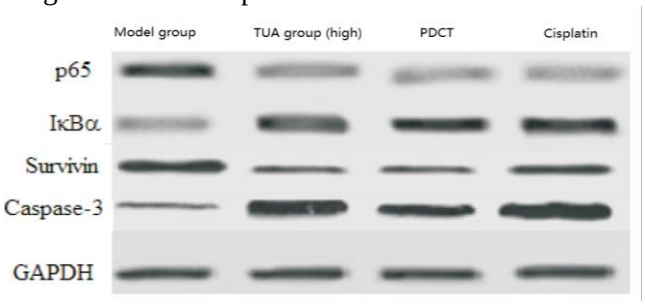


Figure 5. Influence of TUA on expressions of apoptosis-related proteins in tumor.

5. Discussion

As we all know, the urgent problem encountered in the treatment of lung cancer is the wide range of adverse reactions and drug resistance of antitumor drugs used in chemotherapy. It is urgent to adopt new treatment strategies for the diagnosis and treatment of lung cancer. For example, extracting and separating natural products from driven plants with strong anti–lung cancer effect and small toxic and side effects has always been an effective way. In recent years, many studies have demonstrated that TUA isolated from Chinese herbal medicine have extensive antitumor activity. In order to develop more effective anticancer drugs, plant extracts or monomer compounds rich in TUA have attracted more and more interest of researchers. For example, the antitumor effects of ursolic acid, asiatic acid, ilein and a series of its derivatives isolated from Chinese herbal medicine have been confirmed, and some compounds have been applied in clinical trials. However, the antitumor effect of TUA in Actinidia chinensis Radix has not been reported, especially the anti–lung cancer effect of TUA. Therefore, it is necessary to systematically investigate its pharmacological activity. In this study, the results confirmed that TUA had potential inhibitory effect on lung cancer growth *in vivo* (transplanted tumor), and had less toxic and side effects on normal tissues. The experiment also confirmed that its mechanism of inhibiting tumor growth may be related to tumor cell apoptosis through inhibiting NF-κB signaling pathway.

NF-κB is an important transcription factor involved in tumorigenesis associated with inflammatory diseases[9]. NF-κB is generally highly expressed in human lung tumor tissues or tumor cells. High expression of NF-κB is associated with many aspects of tumorigenesis, such as tumor cell growth, anti-apoptotic effect, metastasis, tumor angiogenesis and resistance to chemotherapeutic drugs[10]. The activation of NF-κB is related to tumor cell apoptosis, tumorigenesis and development, so it can inhibit IKKβ/NF-κB signaling pathway to achieve the purpose of treating related tumors[11]. A large number of studies have confirmed that when NF-κB is inhibited, the proliferation of tumor cells is inhibited. At the same time, the cell cycle is also blocked, and the apoptosis is increased gradually[12]. NF-κB combines with DNA target to form homologous or heterodimer and regulates the transcription of downstream genes, resulting in tumor cell proliferation and diffusion[13]. In conclusion, NF-κB plays an important role in the development of innovative antitumor drugs. NF-κB is likely to be a new therapeutic target.

During the research on the prevention and treatment of lung cancer (NSCLCCs), whether *in vivo* or *in vitro*, there are many natural products, such as curcumin[14], vinorelbine[15], Feroniellin A[16], and Minnelide[17]. It has been demonstrated that inhibiting tumor tissue growth and inducing tumor cell apoptosis can be achieved by inhibiting NF-κB pathway. The Tsao's research group showed that Protocatechuic acid (PCA) enables the dose-dependent downregulation of the expression of the NF-κB p50 and NF-κB p65 protein in the lung cancer cell line A549, H3255, and Calu-6[18]. Another study also proved that Bee venom (BV) can regulate NF-κB activity to inhibit the further proliferation of lung cancer A549 and NCI-H460 cell lines[19]. The results of Western blot showed that the expression of p65 protein in transplanted tumors was inhibited after TUA treatment. At the same

time, IκBα protein expression increased. It indicated that the apoptosis of NSCLCCs mediated by TUA is related to the inhibition of NF-κB activity, which is consistent with the above results.

In the development of tumors, cells apoptosis generally plays the role of reversing regulation (which can prevent the rapid proliferation and differentiation of tumor cells). This process is co-regulated by a number of apoptosis promoters and repressors of apoptosis. The ability to stimulate and restore apoptosis of tumor cells is an effective tumor control pathway. Activated NF-κB can inhibit tumor cell apoptosis in a variety of ways, mainly by inducing the expression of anti-apoptotic proteins such as tumor apoptosis protein inhibitory family (IAPS), Bcl-2 family and TRAF family, or inhibiting the expression of apoptosis promoting proteins such as Caspase family. Therefore, inhibition of NF-κB signaling pathway will contribute to the expression of genes involved in tumorigenesis and development, which in turn will accelerate the apoptosis of tumor cells. Survivin, an important member of the IAPs family—tumor apoptosis suppressor proteins, is expressed only in embryonic tissues in non-pathological states but not in adult terminal differentiated tissues. However, in pathological cases, Survivin is expressed in the vast majority of tumor tissues. It is currently believed that the mechanism that Survivin promotes tumor development is probably related to its promoting tumor cell proliferation, inhibiting tumor cell apoptosis, and promoting tumor metastasis and invasion[20]. In recent years, many studies have shown that Survivin upregulates its expression in lung and gastric cancer tissues[21]. The results of this study showed that NCI-H460 cells had decreased Survivin expression in tumor cells after TUA treatment, and that Survivin expression in it was diminished with increasing TUA's concentration. It is speculated that it is due to the regulatory function of gene expression being inhibited, which cannot help tumor cells avoid apoptosis, at the same time blocking the abnormal proliferation of tumor cells and accelerating the further apoptosis of tumor cells. Caspase-3 is an important effector molecule in apoptosis signal transduction pathway, leading to apoptosis[22]. The results also confirmed that the expression level of Caspase-3 in NCI-H460 cells increased after TUA treatment.

The xenograft tumor model of lung cancer NCI-H460 cells in nude mice was prepared to observe the effect of TUA on transplanted tumor in nude mice, so as to provide experimental basis for further study of the anti-lung cancer effect and mechanism of TUA. The results of tumor inhibition experiment and HE staining showed that after treatment with different concentrations of drugs, TUA had a certain inhibitory effect on the transplanted tumor of NCI-H460 nude mice. Compared with the model group, the difference was significant ($P < 0.01$), and the tumor inhibition effect was similar to that of cisplatin and NF-κB, inhibitor PDTC. In order to explore the mechanism of the anti-tumor effect of TUA on NCI-H460 nude mice, in this experiment, immunohistochemistry and Western blot technology were used to detect the expression of the transplanted tumor and the apoptosis-related protein p65, IκBα, Survivin, Caspase-3 in each experimental group. The results also confirmed that the levels of p65 protein expression were decreased. At the same time, the level of protein IκBα expression was increased. Survivin protein expression of proteins associated with apoptosis was inhibited and Caspase-3 protein was upregulated.

In conclusion, this experiment confirmed that TUA has potential inhibitory effect on human lung cancer cell line NCI-H460 by transplanting tumor *in vivo*, and its possible anti-tumor mechanism is mainly to inhibit the NF-κB signaling pathway and the expression of related genes regulated by it, so as to promote tumor cell apoptosis. The results of this study can provide a theoretical basis for the further development of TUA as a clinical anti-lung cancer drug.

Conflict of interest

The authors declare no potential conflicts of interest.

Acknowledgements

Fund project: National Natural Science Foundation of China (81360627); Science and Technology Research Project of Jiangxi Provincial Department of Education (GJJ14694).

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