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ORIGINAL ARTICLE

Linderalactone inhibits human lung cancer growth by modulating the expression of apoptosis-related proteins, G2/M cell cycle arrest and inhibition of JAK/STAT signalling pathway

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Summary

Purpose: *The main purpose of the current research work was to evaluate the antitumor effects of linderalactone in A-549 human lung carcinoma cell line along with the study its effects on apoptosis-related proteins, cell cycle phase distribution and JAK/STAT signalling pathway.*

Methods: *The viability of lung cancer cell line was investigated by MTT assay at varying doses of linderalactone. Apoptosis was detected by using fluorescence microscopy and flow cytometry. Cell cycle analysis was carried out by flow cytometery. The protein expression was examined by western blotting.*

Results: *Linderalactone could inhibit the proliferation of the lung cancer A-549 cells with an IC_{50} of 15 μM. Further investigations indicated the antiproliferative effects of linderalactone are due to apoptosis induction which was further confirmed by Bax and Bcl-2 expression. It also induced G2/M cell cycle arrest which was also associated with alteration of the expression of several important proteins. Furthermore, linderalactone could also suppress the JAK/STAT signalling pathway.*

Conclusions: *In conclusion, linderalactone could be developed as a potential drug candidate against lung cancer provided that further in depth studies are carried out in this direction focusing on its in vivo efficacy.*

Key words: *apoptosis, cell cycle, flow cytometry, linderalactone, lung carcinoma, protein expression*

Introduction

Lung cancer is one of the prevalent causes of cancer-associated deaths across the globe and 85-90% of cases of lung cancer are categorized as non-small cell lung cancer (NSCLC), with most of the patients diagnosed with advanced disease [1]. The treatment strategies for lung cancer are still far from satisfactory and are associated with lots of side effects. Therefore, the need of the hour is to look for novel drug options or to identify novel therapeutic targets for the management of lung cancer [2]. Plants are natural chemical factories producing a wide diversity of chemical scaffolds. The metabolites produced by plants are either primary metabolites without which the plant can't survive and secondary metabolites which are produced as a defense against the biotic and abiotic stresses [3]. Since plants are always exposed to extreme environmental conditions, they have evolved to produce different types of secondary metabolites [4]. These metabolites have been shown to be beneficial in the treatment of human diseases and have been used as antimicrobial and anticancer agents

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besides others [5]. Moreover, these plant secondary metabolites have now gained so much attention that they are screened for their bioactivities, especially anticancer activities, every now and then [6].

Linderalactone is an important sesquiterpene lactone which has been reported to possess immense pharmacological potential and has been shown to inhibit the growth of cancer cells [7].

In the current study the effect of linderalactone was examined against the lung cancer cell line A549. It was found that linderalactone caused considerable inhibition of the proliferation of the human lung cancer A549 cells which was found to be due to the induction of apoptosis. Linderalactone could modulate the expression of apoptosis-related proteins, leading to activation of apoptotic cell death of A549 cells. Furthermore, linderalactone could also cause arrest of the A549 cells at the G2/M checkpoint and as such can prevent the growth of the A549 cells. JAK/STAT pathway is an important pathway that has been reported to be activated in cancer cells [8]. In this study it was observed that linderalactone could inhibit the JAK/STAT pathway in A549 cells, indicating the potential of linderalactone in the treatment of lung cancer.

Methods

Cell line and culture conditions

Lung cancer A549 cell line was obtained from Type Culture Collection of Chinese Academy of Sciences, Shanghai, China and was cultured in RPMI 1640 complete medium containing 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 µg/ml streptomycin), at 37°C in a humidified incubator.

MTT assay

For examination of the proliferation rate, the A549 lung cancer cells were cultured in 96-well plates at 5×10^3 cells per well. The cells were then incubated overnight, followed by the replacement of Dulbecco's modified Eagle's medium (Invitrogen Life Technologies, Massachusetts, USA) with new medium containing linderalactone at different concentrations (0-100 µM) for one day. Thereafter, addition of MTT (0.5 mg/mL) was followed and the cells were incubated for 3-4 hrhs. Finally, the absorbance at 570 nm was assessed by a spectrophotometer.

Apoptosis assay

The nuclear morphology of the A549 cancer cells was assessed by fluorescence microscopy after DAPI staining. Ten fields (200x magnification) with 100 cells/field were selected randomly for estimation of the cells with condensed nuclei. Annexin V/propidium iodide (PI) double staining was used for the determination of apoptotic A549 cancer cells by flow cytometry.

Cell cycle analysis

To check whether linderalactone triggers cell cycle arrest, the treated cells were cultured for 24 hrs, were trypsinized and then fixed with 70% ethanol. Afterwards, staining with PI was performed for 30 min. Finally the cells were subjected to flow cytometry.

Invasion assay

Invasion was evaluated with the help of Matrigel®-coated invasion chambers. The linderalactone treated and untreated A549 cells that reached the lower surface of the membrane were subjected to staining with crystal violet (CV), and images of CV-stained cells were taken. The CV complexes formed were dissolved in 10% acetic acid and the cell invasion was determined by measuring the absorbances of the resultant solutions at 600 nm in a spectrophotometer.

Western blotting

For protein expression analysis the lung cancer cells were subjected to lysis with RIPA buffer and the protein content of each lysate was checked by bicinchoninic acid assay (BCA). Equal volumes of each sample were loaded on the SDS-PAGE. The gel was then transferred to nitrocellulose membrane and incubated with primary antibody for 24 hrs at 4°C and then with secondary antibody at 24°C for about 1 hr. The visualisation of the proteins was performed by enhanced chemiluminescence reagent.

Results

Linderalactone inhibited the growth of A549 cells

The effect of the natural sesquiterpene lactone, linderalactone (Figure 1), was evaluated by MTT assay. The A549 cells were subjected to treatment with varied concentrations of linderalactone and it was found that linderalactone inhibited the growth of A549 cells concentration-dependently. The IC_{50} of linderalactone was 15 µM (Figure 2).

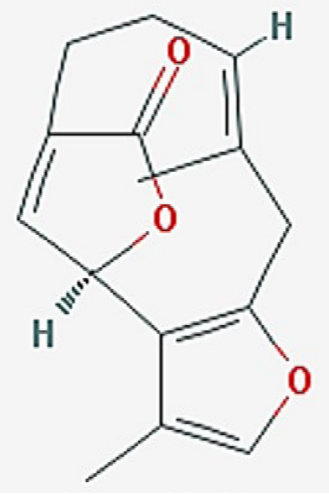

Figure 1. Chemical structure of linderalactone.

Linderalactone induced apoptosis of human A549 cells

Many of the sesquiterpene lactones have been found to induce apoptosis of cancer cells. Given this background, the effects of linderalactone were evaluated on the activation of apoptosis of the human lung cancer cell line A549. The results of the DAPI staining showed that the number of cells with white color nuclei increased, indicative of the apoptosis (Figure 3). Next, the percentage of apoptotic cell populations was determined by annexin V/PI staining and it was found that the percentage of apoptotic A549 cell populations increased with increase in the concentration of linderalactone (Figure 4). Apoptosis was associated with alteration of the expression of several proteins and therefore, we also determined the expression of the apoptosis-related proteins and found that the expression of Bax, cytochrome C and cleaved caspase 9 and 3 in-

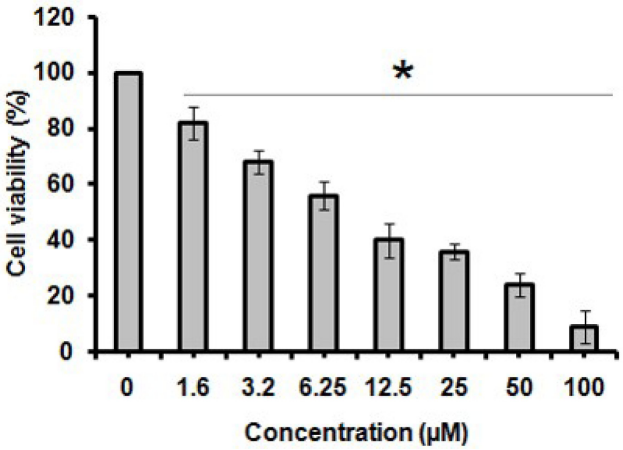

Figure 2. Effect of linderalactone on the viability of A549 cells as determined by MTT assay. The experiments were performed in triplicate (*p< 0.05).

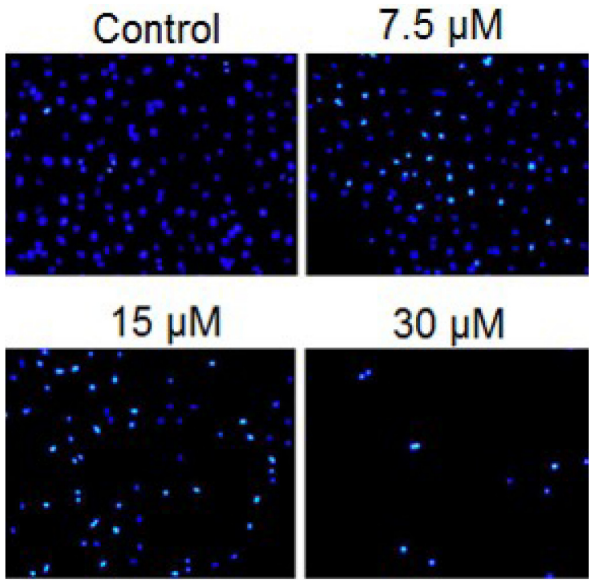

Figure 3. Effect of linderalactone on the apoptosis of A549 cells as determined by DAPI staining. The experiments were performed in triplicate. The Figure depicts that linderalactone induces apoptosis in A549 cells in a dose-dependent manner.

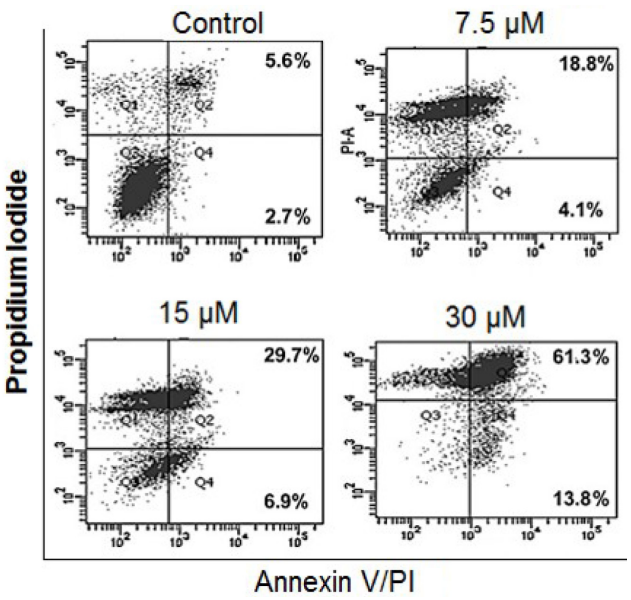

Figure 4. Determination of the percentage of the apoptotic cells as depicted by annexin V/PI staining. The experiments were performed in triplicate. The Figure depicts that the apoptotic A549 cell percentage increases with increase in the concentration of linderalactone.

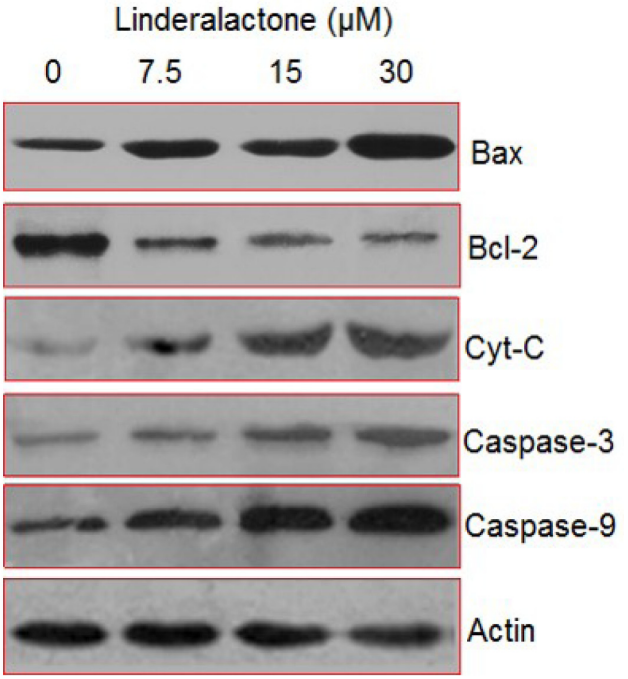

Figure 5. Effect of linderalactone on the cell cycle phase distribution of A549 cells as depicted by Western blotting. The experiments were performed in triplicate. The Figure depicts that linderalactone enhances the expression of Bax, Cyt C, Caspase-3 and 9, while it decreases the expression of Bcl-2 dose-dependently.

creased, while Bcl-2 decreased in a concentration-dependent manner (Figure 5).

Linderalactone triggered G2/M cell cycle arrest

The effect of linderalactone was also assessed on the cell cycle phase distribution of the A549 cells. The cells were subjected to treatment with varied concentrations of linderalactone and assessed by flow cytometry. It was found that upon increasing the concentrations of linderalactone the cells accumulated significantly in the G2 phase. This was mainly due to the arrest of the cells at the G2/M checkpoint, ultimately leading to the G2/M cell cycle arrest (Figure 6).

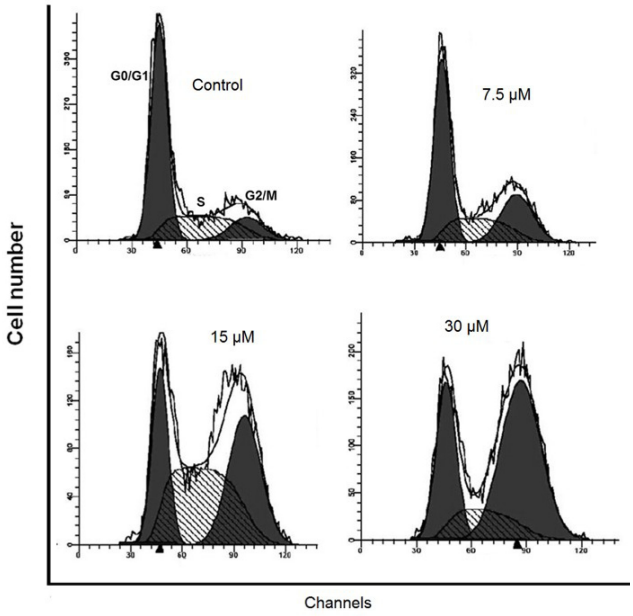

Figure 6. Effect of linderalactone on the cell cycle phase distribution of A549 cells, as depicted by flow cytometry. The experiments were performed in triplicate. The Figure depicts that linderalactone induces G2/M cell cycle arrest of A549 cells dose-dependently.

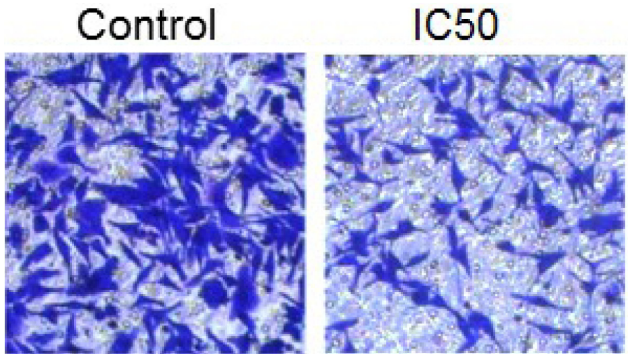

Figure 7. Effect of linderalactone on the cell invasion of A549 cells. The experiments were performed in triplicate. The Figure depicts that linderalactone inhibits the invasion of A549 cells cells at IC_{50}.

Linderalactone affected the invasion of the A549 cells

The effects of linderalactone on the invasion of the A549 cells was assessed by Matrigel invasion assay. The results indicated that linderalactone could inhibit the invasion of the A549 cells at IC_{50} (Figure 7).

Linderalactone inhibited the JAK/STAT pathway in A549 cells

The effect of linderalactone was also evaluated on A549 lung cancer cells. It was observed that linderalactone could inhibit the expression of STAT1, JAK1 and JAK2. Moreover, linderalactone could also inhibit the phosphorylation of pSTAT1, pSTAT-2, pJAK1 and pJAk2. These results clearly indicate that linderalactone inhibits the JAK/STAT pathway A549 lung cancer cells (Figure 8).

Discussion

Lung cancer, being one of the deadliest cancers, is responsible for considerable mortality and

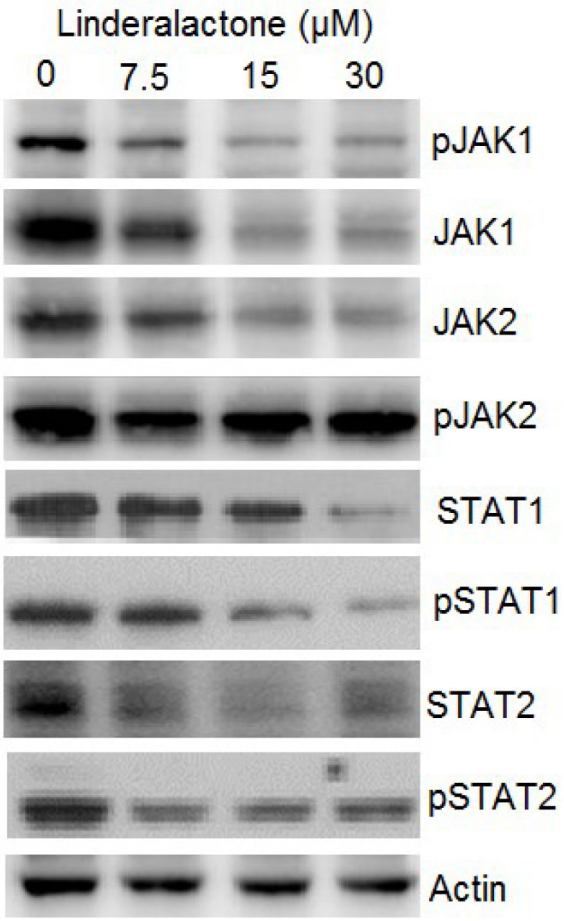

Figure 8. Effect of linderalactone on the expression of JAK/STAT pathway as shown in western blots. The experiments were performed in triplicate. The Figure depicts that linderalactone blocks the JAK/STAT pathway in A549 cells.

morbidity world over [9]. However, its treatment options are limited and hence there is pressing need to explore novel drug options and/or to look for novel therapeutic drug targets [10]. Plants produce a number of compounds that exhibit anticancer properties. These metabolites are considered safer for human administration. Moreover, plants have served as source of anticancer agents in the past and are likely to continue as important sources of drugs in the future [11]. Linderalactone is an important sesquiterpene lactone that has been reported to inhibit the growth of several types of cancer cells [12]. However, the anticancer effects of linderalactone have not been evaluated against lung cancer cell lines. It was found that linderalactone exerts considerable anticancer effects on the A549 lung cancer cells and reduces their viability. Linderalactone exhibited IC_{50} 15 μM against the A549 cancer cells. It has been found that several of plant-derived lactones exert growth inhibitory effects on cancer cells by prompting apoptosis [13]. Apoptosis eliminates the harmful cells from the body and a number of the currently used anticancer drugs induce apoptotic cell death [14]. Herein we also observed that linderalactone induces apoptosis of the A549 cells which was also associated with modulation of apoptosis-related proteins. In addition to apoptosis, arrest of cells during cell division curbs the growth of cancer cells [15]. In this study we found that linderalactone causes G2/M cell cycle arrest of A549 cells.

Most of the metastatic cancers are difficult to treat [16]. However, in the present study we observed that linderalactone could inhibit the metastatic process of cancer cells. JAK/STAT signalling pathway is an important pathway and has been considered as an important therapeutic target for the treatment of several cancers [17]. We found that linderalactone could inhibit this pathway, proving the potential of linderalactone in the treatment of lung cancer.

Conclusion

Taken together, we conclude that linderalactone is an important plant-derived natural product which exerts anticancer effects on the A549 lung cancer cells by triggering apoptosis and cell cycle arrest. Besides, linderalactone could suppress cell invasion and inhibit the JAK/STAT pathway. Hence, this molecule may prove beneficial for the treatment of lung cancer and warrants further investigation.

Conflict of interests

The authors declare no conflict of interests.

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