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Research Article

$K_5BW_{12}O_{40}$ Induces the Apoptosis of A549 Cells by Regulating Caspase-3

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Objective. The purpose of the study is to explore the effect of $K_5BW_{12}O_{40}$, a polyoxometalate (POM), on the apoptosis of A549 cells and its underlying mechanism and to analyze the potential therapeutic effect of $K_5BW_{12}O_{40}$ in non-small-cell lung cancer. *Materials and Methods*. A549 cells were treated with different concentrations of $K_5BW_{12}O_{40}$ (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml). The proliferation of A549 cells treated with different concentrations of $K_5BW_{12}O_{40}$ was detected by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyl tetrazolium bromide). The apoptosis of A549 cells induced by $K_5BW_{12}O_{40}$ was detected by flow cytometry. Western blot was used to detect the changes in Bax and caspase-3 protein levels in A549 cells induced by $K_5BW_{12}O_{40}$. *Results*. As the dose of the $K_5BW_{12}O_{40}$ increases, the cell viability of A549 cells gradually decreases. The results of flow cytometry showed that the apoptotic rate of A549 cells increased with the increase of $K_5BW_{12}O_{40}$ concentration. Western blot results showed that the expression of the apoptosis marker protein caspase-3 was increased in the three groups treated with $K_5BW_{12}O_{40}$ whereas the protein level of Bax did not change significantly in A549 cells treated with $K_5BW_{12}O_{40}$. *Conclusions*. $K_5BW_{12}O_{40}$ increases the apoptotic rate of A549 cells by upregulating caspase-3.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths throughout the world [1]. According to histopathological classification, lung cancer can be divided into two categories, one is small cell lung cancer and the other is non-small-cell lung cancer (NSCLC) [2]. As a type of lung cancer, non-small-cell lung cancer is a common malignant tumor with high mortality. In recent years, new progress has been made in surgery, radiotherapy, chemotherapy, and targeted therapy for non-small-cell lung cancer patients, and traditional chemotherapy has been widely used in the treatment of non-small-cell lung cancer, but its severe side effects and drug resistance limit its clinical application [3]. Currently, immunotherapy is increasingly being used for lung cancer patients. For example, PD-L1 suppresses CD8 cytotoxic immune responses and produces antitumor immune responses; however, there is an urgent need to develop a novel and universal biomarker to assess PD-L1 expression [4]. Surgical resection is the preferred option for treating NSCLC. Besides, chemotherapy and radiotherapy are performed for NSCLC patients who cannot be operated [5]. Although the potential chemotherapeutic efficacy of novel compounds on NSCLC has been confirmed in many studies, the sensitivity of high-grade NSCLC to chemotherapeutic drugs remains poor, and the exact mechanism of such a phenomenon has not been fully clarified [6]. Therefore, alternative therapeutic drugs that can effectively treat lung cancer have recently attracted attention [7].

There have been many reports that confirmed that polyoxometalates (POMs) can inhibit the growth of tumor cells due to their structural diversity and significantly

reduced toxicity [8]. [9] Polyoxometalates have adjustable structural charge and size and possess the potential to produce organic-inorganic hybrids [10]. Because of these unique structures and excellent properties, polyoxometalates have attracted attention, and they have very important applications in the fields of catalysis, medicine, and functional materials [11]. Several studies have shown that the POMs can promote the apoptosis of A549 cells, such as $K_9(C_4H_4FN_2O_2)_2Nd(PW_{11}O_{39})_2{\cdot}25H_2O$ [12] and SbW9 [9], but the role of $K_5BW_{12}O_{40}$ in lung cancer is still unclear, and the POM used in this paper is structurally stable. Therefore, $K_5BW_{12}O_{40}$, as a polyoxometalate, is worthy of research in the treatment of cancer. In this study, detecting the effect of $K_5BW_{12}O_{40}$ on the proliferation and apoptosis of A549 cells and its molecular mechanism provides a new treatment method and strategy for the treatment of lung cancer.

2. Materials and Methods

2.1. Materials and Cells. $K_5BW_{12}O_{40}$ was presented by Professor Yaguang Chen of Northeast Normal University. In this paper, we want to investigate the effect of $K_5BW_{12}O_{40}$ on non-small-cell lung cancer, so the non-small-cell cancer cell line A549 was used. The NSCLC cell line A549 was from the Department of Immunology, College of Basic Medical Sciences, Jilin University. A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM, BI, Israel) supplemented with 10% fetal bovine serum (FBS, BI, Israel), in an incubator at 37°C and 5% CO_2. Cells were splitted every other day.

2.2. Cell Intervention. A549 cells were inoculated into a 96-well plate (8000 cells per well) and treated with $K_5BW_{12}O_{40}$ in different concentrations (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml). The concentration of polyoxometalates in this article refers to related articles. After treatment for 48 h, MTT was added for incubation at 37°C for another 4 h, and the absorbance was read at 450 nm every hour.

2.3. Cell Imaging. A549 cells were inoculated into a 96-well plate (8000 cells per well) and treated with $K_5BW_{12}O_{40}$ in different concentrations (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml). After treatment for 48 h, pictures were taken using an inverted microscope (CKX53, OLYMPUS, Japan).

2.4. Flow Cytometry Analysis. Apoptosis of A549 was determined using the Annexin V-PE apoptosis kit (Sungene Biotech, China) and following their instructions. All of the samples were analyzed using a FACSCalibur cytometer (BD, the USA).

2.5. Protein Isolation and Western Blot Analysis. A549 cells were harvested and incubated with ice-cold RIPA lysis buffer (Beyotime, China) and protease inhibitor cocktail tablets (Roche, USA). After electrophoresis and electrotransfer to a PVDF membrane, anti-caspase-3 (1 : 2000, CST, USA) and anti-β-actin (1 : 5000, Proteintech, China) were incubated with the PVDF membrane at 4°C overnight. A chemiluminescent substrate (ECL, GE Healthcare, USA) was used for protein visualization.

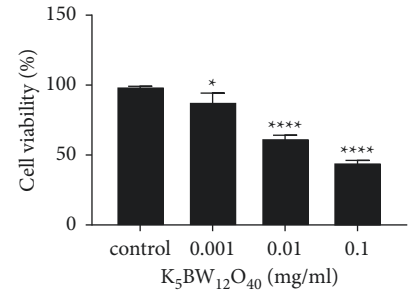


Figure 1: Effect of $K_5BW_{12}O_{40}$ on the survival of A549 cells. Control: control group. $^{*}P < 0.05$, $^{****}P < 0.0001$. There is a statistically significant difference compared with control group.

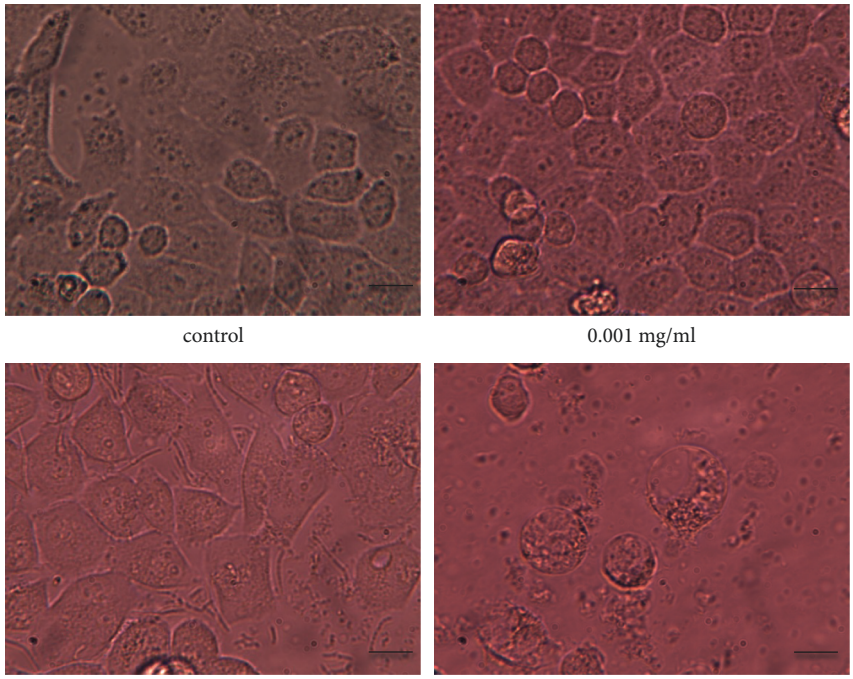
2.6. Statistical Analysis. The data were presented as the mean ± standard deviation with GraphPad Prism software 8.0. Statistical Product and Service Solutions (SPSS) 16.0 software (IBM, Armonk, NY, USA) was used for the analysis of all data. The data between the two groups were compared using the t-test. $P < 0.05$ was considered significant.

3. Results

3.1. $K_5BW_{12}O_{40}$ Inhibited the Proliferation of A549 Cells in a Concentration-Dependent Manner. After treatment of A549 cells with $K_5BW_{12}O_{40}$ in different concentrations (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml) for 48 h, the cell proliferation in each group was detected via the MTT assay. As shown in Figure 1, $K_5BW_{12}O_{40}$(0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml) could significantly inhibit the proliferation of A549 cells in a concentration-dependent manner ($P < 0.05$).

3.2. $K_5BW_{12}O_{40}$ Induced Apoptosis of A549 Cells. After treatment of A549 cells with $K_5BW_{12}O_{40}$ in different concentrations (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml) for 48 h, an image of the cell was taken using an inverted microscope (Figure 2), The result demonstrated that $K_5BW_{12}O_{40}$ induced apoptosis of A549 cells. In order to further understand the effect of $K_5BW_{12}O_{40}$ on the apoptosis of A549 cells, we performed cell flow cytometry. The results of flow cytometry showed that the $K_5BW_{12}O_{40}$ exerted a significant proapoptotic effect on A549 cells. In different concentration groups, the apoptosis rates of the control group and the $K_5BW_{12}O_{40}$ group were 12.9, 43.0, 34.7, and 26.5 ($P < 0.05$) (Figure 2), which indicates that the $K_5BW_{12}O_{40}$ can promote the apoptosis of A549 cells.

3.3. Influence of $K_5BW_{12}O_{40}$ on the Apoptosis-Related Proteins in A549 Cells. In order to detect how $K_5BW_{12}O_{40}$ affects cell apoptosis, we tested the expression of apoptosis-related



(a)

Figure 2: Continued.

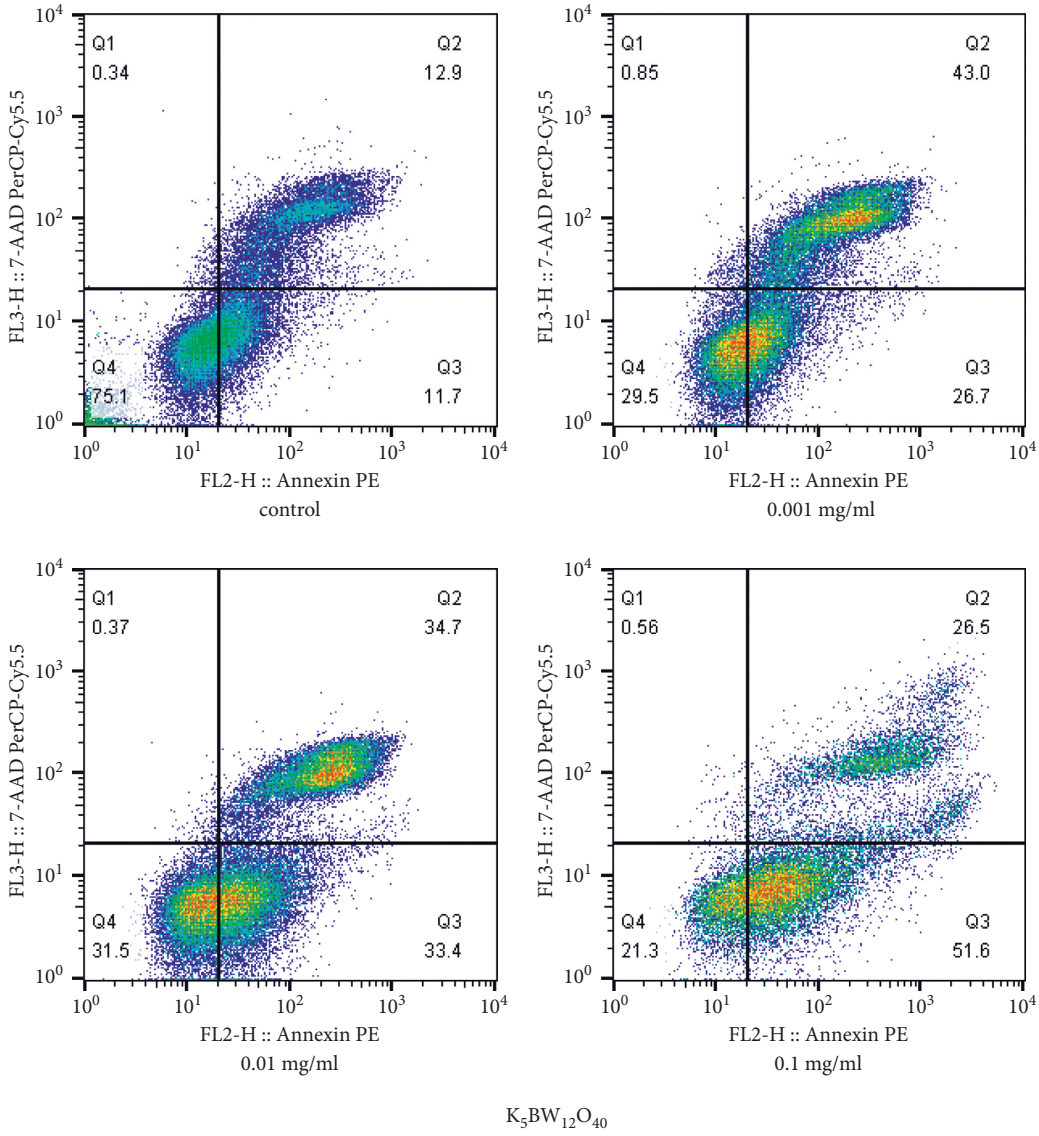


Figure 2: Polyoxometalate $K_5BW_{12}O_{40}$ promotes the apoptosis of A549 cells. (a) The image of A549 cells. (b) A549 cells were treated with $K_5BW_{12}O_{40}$ for 48 h and then stained using the Annexin V-FITC/PI detection kit and detected by FCM. Annexin V-FITC single positive cells are early apoptotic cells, Annexin V-FITC and PI double positive cells are necrotic or late apoptotic cells, and PI single positive cells are naked nuclear cells. Original magnification × 40, scale bar = 10 μm.

proteins. The results of western blotting showed that after treatment with $K_5BW_{12}O_{40}$, the expression of proapoptotic protein caspase-3 was upregulated in A549 cells, suggesting that the inducible effect of $K_5BW_{12}O_{40}$ on the apoptosis of A549 cells is dependent on the Bcl-2 pathway (Figure 3).

4. Discussion

Lung cancer is the most common cause of cancer death in the world [13]. Lung cancer is mainly divided into two types, namely, SCLC and NSCLC [14]. Although great progress has been made in the diagnosis and treatment of lung cancer, the treatment and prognosis of lung cancer still have not achieved good results [3]. Due to the high prevalence of lung cancer and the rising number of deaths, research on cancer control and treatment has been ongoing. There is evidence that the occurrence and prognosis of lung cancer are closely related to the proliferation and apoptosis of lung cancer cells [15]. Therefore, exploring how to inhibit the proliferation of lung cancer cells and promote their apoptosis is of great significance for the treatment of lung cancer.

Although many efforts have been made in cancer treatment, only limited achievements have been achieved in clinical practice. Cisplatin has been widely applied in

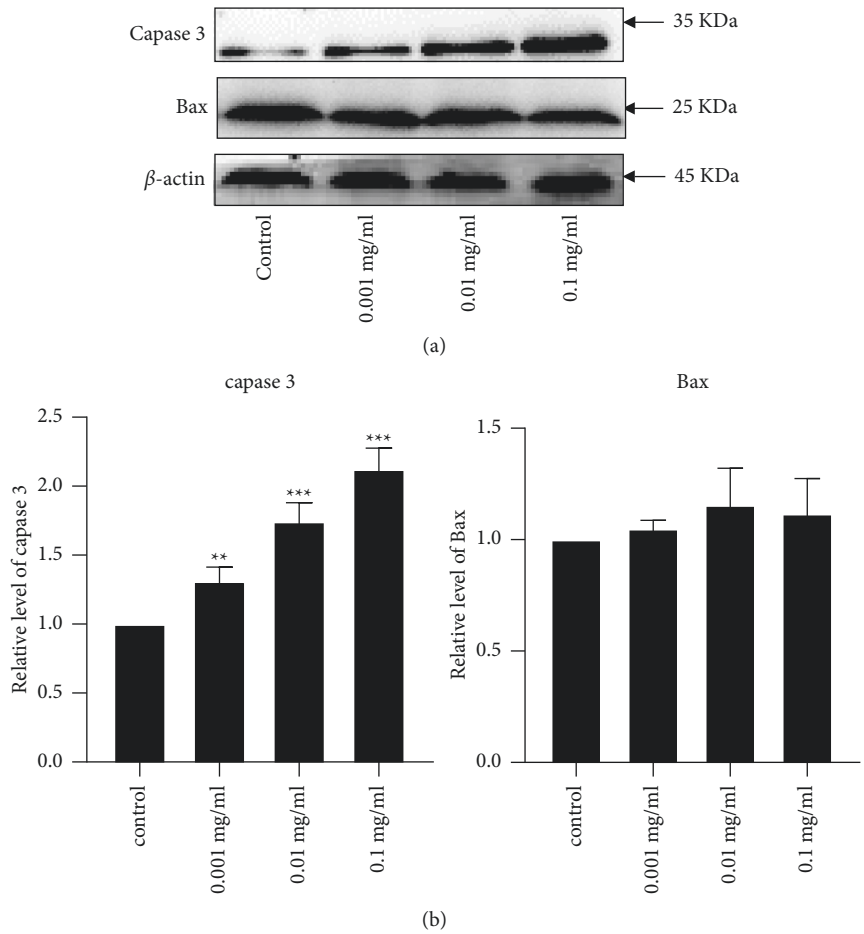


FIGURE 3: Effect of $K_5BW_{12}O_{40}$ on the apoptosis-related protein of A549 cells. Apoptosis determined by western blot. (a) Protein expressions of capase3. Bax in A549 cells treated with different doses of (0, 0.001, 0.01, and 0.1 mg/ml) for 48 h. (b) Data are presented as the mean ± SD; there is a statistically significant difference compared with control group. $^{**}P < 0.01$. $^{***}P < 0.001$.

chemotherapy for tumors, but it has certain curative effects, patients with lung cancer are resistant to cisplatin-based therapies [16 ,17], which has become a major obstacle in cancer treatment [18]. Due to the emergence of more and more drug resistance, the effect of cancer is not ideal. Therefore, there is an urgent need to find new targets that can reduce drug resistance and increase the sensitivity of tumor cells to chemotherapy.

Over recent decades, POMs have attracted more and more attention due to their good redox activities and outstanding electron and proton transport capacities [19], and it has been proven that they can inhibit the development of cancer [8]; polyoxometalates have been widely used as antitumor drugs in medicine [20, 21]. The anticancer effects of POMs were first mentioned in 1965. Mukherjee et al. proposed that a mixture of polyacid compounds and caffeine could eliminate tumors in patients with gastrointestinal cancer [22]. Many previous studies have shown that POMs exert antitumor effects in various malignancies such as breast, lung, ovarian, and pancreatic cancers by regulating cell proliferation, invasion, and migration [23]. POMs have antitumor activity due to their high affinity for important enzymes such as kinases [24], actin [25], and P-type ATPases [26]. SbW9 can inhibit the proliferation of NSCLC cells and promote the apoptosis of NSCLC cells, and the mechanism may be mediated by the PTEN/AKT signaling pathway [9]. $[NH_3Pri]^{6-}[H_XMo_7O_{24}]$ significantly inhibited the growth of Meth A sarcoma in athymic nude mice [27], and polyoxometalate has also been identified as an inhibitor of protein kinase CK2, a multifunctional kinase that is dysregulated in many cancers. In addition, FNdPW, a polyoxometalate that includes rare earth elements, has antiproliferative effects on A549 cells by causing apoptosis [12].

In this study, A549 cells were stimulated with the polyoxometalate $K_5BW_{12}O_{40}$ in different doses in in vitro experiments. It was found in the MTT assay that the $K_5BW_{12}O_{40}$ significantly inhibited the proliferation. At the same time, the apoptosis in each group was further detected via flow cytometry. The results showed that $K_5BW_{12}O_{40}$ could well induce the apoptosis of A549 cells. Furthermore, the western blotting showed that the inducible effect of $K_5BW_{12}O_{40}$ on the apoptosis of A549 cells may be dependent on the caspase-3 pathway.

To sum up, $K_5BW_{12}O_{40}$ treatment upregulated the protein level of caspase-3 and activated apoptotic pathways

in A549 cells. Our study may provide a new idea and therapeutic foundation for the clinical treatment of NSCLC.

5. Conclusion

This study reveals that $K_5BW_{12}O_{40}$ promotes A549 cell apoptosis by upregulating casepse-3.

Data Availability

The data that support the finding of this study are available in this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

WC, DL, LL, and SL made the study design; LL and SL conducted the study; LL, SL, and RL wrote the manuscript; WD and CW attended the manuscript revision. All the authors agreed with the final manuscript. Liping Liu and Shanshan Liu contributed equally to this work.

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