Research Article

K₅BW₁₂O₄₀ 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 K₅BW₁₂O₄₀, a polyoxometalate (POM), on the apoptosis of A549 cells and its underlying mechanism and to analyze the potential therapeutic effect of K₅BW₁₂O₄₀ in non-small-cell lung cancer.

Materials and Methods. A549 cells were treated with different concentrations of K₅BW₁₂O₄₀ (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 K₅BW₁₂O₄₀ was detected by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyl tetrazolium bromide). The apoptosis of A549 cells induced by K₅BW₁₂O₄₀ 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 K₅BW₁₂O₄₀.

Results. As the dose of K₅BW₁₂O₄₀ 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 K₅BW₁₂O₄₀ concentration. Western blot results showed that the expression of the apoptosis marker protein caspase-3 was increased in the three groups treated with K₅BW₁₂O₄₀ whereas the protein level of Bax did not change significantly in A549 cells treated with K₅BW₁₂O₄₀.

Conclusions. K₅BW₁₂O₄₀ 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
2. Materials and Methods

2.1. Materials and Cells. K5BW12O40 was presented by Professor Yaguang Chen of Northeast Normal University. In this paper, we want to investigate the effect of K5BW12O40 on the treatment of lung cancer. In this study, detecting the effect of K5BW12O40 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.

2.2. Cell Intervention. A549 cells were inoculated into a 96-well plate (8000 cells per well) and treated with K5BW12O40 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 K5BW12O40 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.

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. K5BW12O40 Inhibited the Proliferation of A549 Cells in a Concentration-Dependent Manner. After treatment of A549 cells with K5BW12O40 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, K5BW12O40 (0 mg/ml, 0.001 mg/ml, 0.01 mg/ml, and 0.1 mg/ml) for 48 h, the concentration groups, the apoptosis rates of the control group and the K5BW12O40 group were 12.9, 43.0, 34.7, and 26.5 (P < 0.05) (Figure 2), which indicates that the K5BW12O40 can promote the apoptosis of A549 cells.

3.2. K5BW12O40 Induced Apoptosis of A549 Cells. After treatment of A549 cells with K5BW12O40 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 K5BW12O40 induced apoptosis of A549 cells. In order to further understand the effect of K5BW12O40 on the apoptosis of A549 cells, we performed cell flow cytometry. The results of flow cytometry showed that the K5BW12O40 exerted a significant proapoptotic effect on A549 cells. In different concentration groups, the apoptosis rates of the control group and the K5BW12O40 group were 12.9, 43.0, 34.7, and 26.5 (P < 0.05) (Figure 2), which indicates that the K5BW12O40 can promote the apoptosis of A549 cells.

3.3. Influence of K5BW12O40 on the Apoptosis-Related Proteins in A549 Cells. In order to detect how K5BW12O40 affects cell apoptosis, we tested the expression of apoptosis-related
Figure 2: Continued.
proteins. The results of western blotting showed that after treatment with K₅BW₁₂O₄₀, the expression of proapoptotic protein caspase-3 was upregulated in A549 cells, suggesting that the inducible effect of K₅BW₁₂O₄₀ 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

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**Figure 2:** Polyoxometalate K₅BW₁₂O₄₀ promotes the apoptosis of A549 cells. (a) The image of A549 cells. (b) A549 cells were treated with K₅BW₁₂O₄₀ 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.
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]. [NH₃Pri]₆[HₓMo₇O₂₄] 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 K₅BW₁₂O₄₀ in different doses in vitro experiments. It was found in the MTT assay that the K₅BW₁₂O₄₀ significantly inhibited the proliferation. At the same time, the apoptosis in each group was further detected via flow cytometry. The results showed that K₅BW₁₂O₄₀ could well induce the apoptosis of A549 cells. Furthermore, the western blotting showed that the inducible effect of K₅BW₁₂O₄₀ on the apoptosis of A549 cells may be dependent on the caspase-3 pathway.

To sum up, K₅BW₁₂O₄₀ treatment upregulated the protein level of caspase-3 and activated apoptotic pathways.
References


