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Original Research

Ginsenoside compound K inhibits growth of lung cancer cells via HIF-1α-mediated glucose metabolism

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Abstract: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths. Compound K, an active metabolite of ginsenosides, is reported to exhibit anti-cancer property in various types of human malignancies. The present study investigated the role of compound K on glucose metabolism in NSCLC cells and its underlying mechanism. Our study found that compound K dose-dependently inhibited the cell viability of NSCLC cells. Moreover, administration with compound K decreased glucose uptake and lactate secretion under normoxic and hypoxic conditions. Consistently, the expression of key enzymes (HK II, PDK1 and LDHA) involved in glucose metabolism were inhibited in compound K-treated tumor cells. In addition, compound K inhibited the expression of HIF-1α and its downstream gene GLUT1. On the contrary, overexpression of HIF-1α elevated metabolic reactions and partly attenuated the inhibitory role of compound K on NSCLC cell growth. These results demonstrate that compound K suppresses NSCLC cell growth via HIF-1α mediated metabolic alteration, contributing to novel anticancer therapy by targeting glucose metabolism.

Key words: Non-small cell lung cancer; Compound K; HIF-1α; Aerobic glycolysis.

Introduction

Lung cancer is the leading cause of cancer-related death, in which 80% of lung cancers are non-small cell lung cancer (NSCLC) with poor therapeutic efficacy when diagnosed (1,2). Despite of the great improvement achieved in cancer therapy, approximately 40% of patients with NSCLC present with advanced-stage disease, for which 5-year survival rates are in the region of 2% (3). Therefore, it is urgent to develop novel therapeutic approach for clinical treatment of NSCLC.

As a major metabolite of ginsenosides, compound K (20-O-(b-D-glucopyranosyl)-20(S)-protopanaxadiol) is widely studied for its anti-inflammatory response, anti-cancer and neuroprotective activities. It has been reported that compound K could inhibit the proliferation, migration and induce apoptosis in several human malignancies such as pulmonary adenocarcinoma, myeloid leukemia, hepatoma cells, and gastric adenocarcinoma (4-6). Furthermore, compound K also possesses chemosensitive activity against chemical carcinogens, and impairs tumor growth and metastasis *in vivo*, which is mediated by 12-O-tetradecanoylphorbol-13-acetate-induced cyclooxygenase-2 expression (7,8). These findings reveal the great potential of compound K in clinical treatment of human cancers.

In tumor cells, metabolic reprogramming is believed to be one of the hallmarks in parallel with chronic inflammation, genomic instability, immune escape, etc. (9). Aerobic glycolysis, termed the Warburg effect, is a characteristic metabolic feature of cancer cells, in which tumor cells tend to produce large amounts of lactate from glucose, regardless of the available oxygen levels (10). Based on this theory, targeting metabolic reprogramming of tumors may be a novel therapeutic approach for human cancers. Indeed, studies have reported that compound K is active in biological systems and inhibits glucose uptake in cancer cells, suggesting that compound K may be pivotal in metabolic reprogramming of malignant cells (11). However, little is known about the metabolic regulation by compound K in NSCLC cells. Therefore, the present study aimed to identify the potential therapeutic effects of compound K on human lung cancer. Furthermore, we investigated the molecular mechanism by which compound K exhibited such anti-cancer effects.

Materials and Methods

Cell culture

Human lung cancer cell lines, including NCI-H460, A549 and NCI-H1299 cell lines, and the HBE human bronchial epithelial cell line were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum,

100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen). All cells were maintained at 37°C in 5% CO_2 incubator.

Cell viability assay

A total of $1x10^4$ cells for each well were seeded in 48-well plates for overnight. The medium was replaced with fresh medium with or without compound K at the indicated concentrations (10, 20, 30, 40, and 50 μg/ml). Cell viability was measured using the 3-[4,5 dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Absorbance was measured at 570 nm using a Microplate Reader.

Real time PCR

Total RNAs were isolated from cultured cells using RNeasy mini-kit (Qiagen, Germany) and reversely transcribed into cDNA using TaKaRa RNA PCR Kit (TaKaRa, Japan) at the following conditions: 42°C for 30 min and 99°C for 5 min. Real time PCR was performed on ABI 7600 system with the following conditions: 95°C for 1 min followed by 40 cycles of 95°C for 30 s, annealing for 30 s and 72°C for 1 min. The primer sequences were shown as follows: HK II: (sense) 5'-AATGGAGCGAGGTCTGAGCAA-3'; (antisense) 5'-CTCCAAGGTCCAAGGCCAAG-3'. PDK1: (sense) 5'-CATGTCACGCTGGGTAATGAGG-3'; (antisense) 5'-GAGGTCTCAACACGA GGTCTTGG-3'. LDHA: (sense) 5'-CATGGCCTGTGCCATCAGTATC-3'; (antisense) 5'-TGCCAG AGACAATCTTTGGTGTTC-3'. HIF-1α: (sense) 5'-CAGCCGCTGGAGACACAATC-3'; (antisense) 5'-TTTCAGCGGTGGGTAATGGA-3'. GLUT1: (sense) 5'- CCAGCCACACAAAACCACTG-3'; (antisense) 5'- GCGAAAATGGATACCGCCAC-3'. β-actin: (sense) 5'-CTGTCCCTGTATGCCTCT-3'; (antisense) 5'-ATGTCACGCACGATTTCC-3' .All reactions were performed in triplicate. The relative expression of each gene was calculated by using the comparative CT method.

Measurement of glucose uptake

Glucose consumption was measured by using an Amplex Red Glucose/Glucose Oxidase kit (Invitrogen, Eugene, USA) according to the manufacturer's protocol. In brief, cells were treated with compound K for 24 h. Then, the culture media supernatant was discarded and the cells were washed in a glucose-free medium. The concentration of glucose uptake was determined by using a microplate spectrophotometer (Thermo Fisher Scientific, USA).

Measurement of lactate production

Lactate generation was measured by using a Lactic Acid Assay Kit (KeyGen Biotech, Nanjing, China) according to the manuscripts' instruction. In brief, cells were treated with compound K for 24 h. Then, the culture media supernatant was collected for the detection of lactate generation. The absorbance was determined by microplate spectrophotometer (Thermo Fisher Scientific, USA).

Western blot

Cancer cells were lysed in cell lysis buffer (Cell Signaling, Danvers, MA, USA). The protein samples were subject to electrophoresis using SDS-PAGE and then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies overnight, and then incubated with appropriate horseradish peroxides–conjugated secondary antibodies for 2 h. Finally, protein bands were detected using an Enhanced Chemiluminescence kit (Pierce, Rockford, IL).

Statistical analysis

Experimental data were presented as means ± SD and treated for statistics analysis by SPSS 19.0 software (Chicago, IL, USA). Comparison between groups was made using ANOVA and statistically significant difference was defined as $P<0.05$.

Results

Compound K induces cell death in lung cancer cells

Firstly, lung cancer cell lines (NCI-H460, A549 and NCI-H1299) and the HBE human bronchial epithelial cell line were treated with different concentrations of compound K to investigate the cytotoxic activity of compound K against these cells. After 24h, cell viability was measured using the MTT assay. Consequently, compound K inhibited the cell viability of NCI-H460 lung cancer cells in a dose-dependent manner (Figure 1A). In addition, we observed a similar inhibitory effect of compound K on A549 (Figure 1B) and NCI-H1299 cell lines (Figure 1C). However, the proliferation of HBE bronchial epithelial cells was not affected by compound K at low concentrations (10, 20, 30, 40 μg/ml) (Figure 1D). Therefore, these data suggest that compound K could inhibit the proliferation of NSCLC cells. And 40 μg/ml of compound K was selected for further study.

Compound K suppresses glucose metabolism in lung cancer cells

Tumor cells are characterized by elevated glucose uptake and lactate production, regardless of the avai-

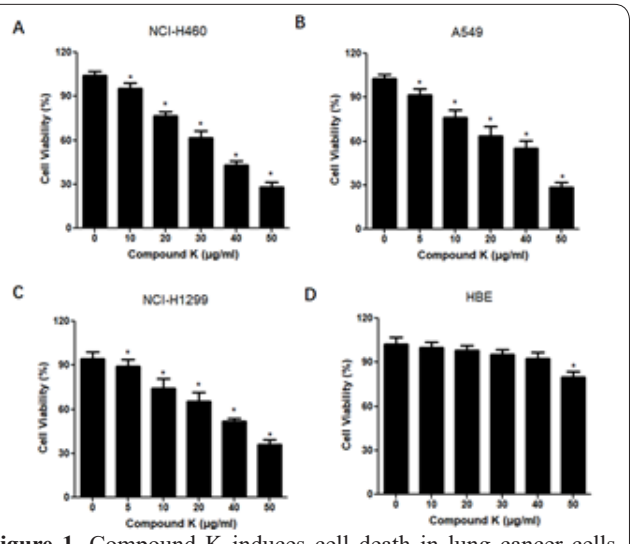

Figure 1. Compound K induces cell death in lung cancer cells. Three human NSCLC cell lines including NCI-H460 (A), A549 (B), NCI-H1299 (C) and HBE (D) were treated with compound K at indicated concentrations. MTT assay was performed to measure the cell viability of tumor cells. Results are expressed as mean ± SD, * $P<0.05$.

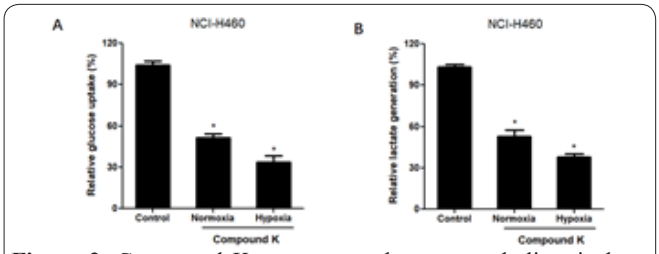

Figure 2. Compound K suppresses glucose metabolism in lung cancer cells. NCI-H460 cells were treated with or without compound K under normoxic or hypoxic conditions. The glucose uptake (A) and lactate generation (B) were detected in tumor cells under normoxic (21% O_2) or hypoxic (5% O_2) conditions. Results are expressed as mean ± SD, * P <0.05.

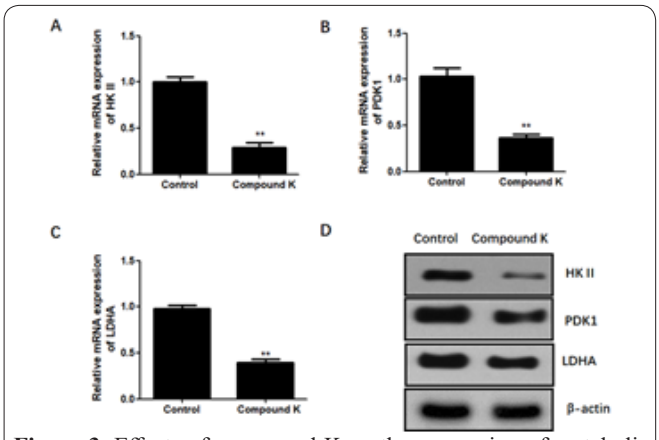

Figure 3. Effects of compound K on the expression of metabolic enzymes in lung cancer cells**.** NCI-H460 cells were treated with or without compound K for 24 h. Then, real time PCR was performed to detect the mRNA levels of HK II (A), PDK1 (B) and LDHA (C). Moreover, western blot was used to measure the protein expression of HK II, PDK1 and LDHA (D). Results are expressed as mean ± SD, ** P <0.01.

lable oxygen levels. Thus, we detected the glucose metabolism of lung cancer cells treated with or without compound K under normoxic (21% O_2) or hypoxic (5% O_2) conditions. As a result, compound K treatment inhibited the glucose consumption and lactate production in NCI-H460 lung cancer cells under normoxic conditions; and the differences are further increased under hypoxic conditions (Figure 2A and B). In addition, we examined the expression levels of key enzymes (HK II, PDK1, LDHA) involved in the glucose metabolism reactions. Consequently, compound K administration obviously decreased the mRNA levels of HK II, PDK1 and LDHA (Figure 3A-C). Moreover, western blot analysis also revealed the reduction of HK II, PDK1 and LDHA at the protein levels in lung cancer cells after treatment with compound K (Figure 3D). These results supported the observation that compound K suppresses lung cancer cell growth by inhibiting glucose metabolism levels.

Compound K reduces HIF-1α expression of in lung cancer cells

It is well known that HIF-1α functions as a critical transcriptional regulator of glycolysis. We further detected the expression of HIF-1α in NCI-H460 lung cancer cells. Real time PCR showed that compound K administration suppressed the mRNA and protein levels of HIF-1α in NCI-H460 cells both under normoxic and hypoxic environment (Figure 4A and C). In addition, the expression of GLUT1, a HIF-1α-dependent enzyme, was also down-regulated in lung cancer cells after compound K treatment (Figure 4B and C). Collectively, these data suggest that compound K could inhibit the expression of HIF-1α, which leads to decreased glucose metabolism in lung cancer cells.

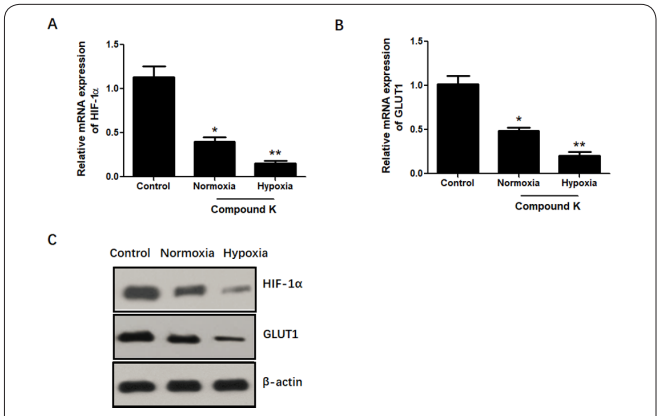

Figure 4. Compound K reduces HIF-1α expression of in lung cancer cells**.** NCI-H460 cells were treated with or without compound K under normoxic (21% O_2) or hypoxic (5% O_2) conditions. Then, real time PCR was performed to detect the mRNA levels of HIF-1α (A) and GLUT1 (B). In addition, western blot was used to measure the protein expression of HIF-1α and GLUT1 (C). Results are expressed as mean ± SD, ** P <0.01.

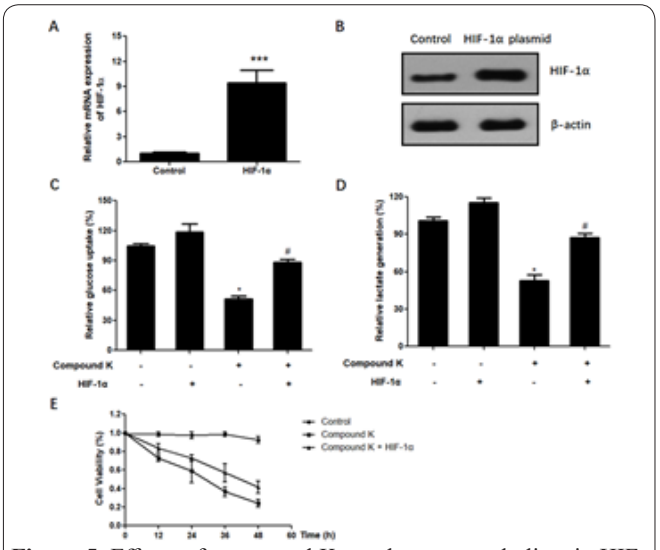

Figure 5. Effects of compound K on glucose metabolism in HIF-1α over-expressed lung cancer cells. NCI-H460 cells were transfected with HIF-1α-encoding plasmids and treated with or without compound K. Real time PCR (A) and western blot (B) were used to measure the mRNA and protein expression of HIF-1α. Alteration of glucose uptake (C), lactate generation (D) and cell viability (E) were measured in NCI-H460 cells. Results are expressed as mean ± SD, ** P <0.01.

Overexpression of HIF-1α attenuates the inhibitory effects of compound K on glucose metabolism

We further investigated the role of HIF-1α in the metabolic regulation of compound K. As shown in Figure 5A and B, HIF-1α was upregulated by transfecting HIF-1α plasmid in NCI-H460 cells. In addition, we detected the role of HIF-1α on compound K- mediated metabolic changes. Consequently, up-regulation of HIF-1α elevated the glucose uptake and lactate product (Figure 5C and D). Furthermore, overexpression of HIF-1α also attenuated the inhibitory effect of compound K on lung

cancer cell growth (Figure 5E). Taken together, these results suggest that the regulation of compound K on glucose metabolism and cell growth is mediated by the involvement of HIF-1α pathway.

Discussion

NSCLC accounts for 80% of lung cancers with poor therapeutic efficacy when diagnosed. Cell death is a complex process that is coordinately or independently mediated by various cellular and molecular events. Thus, therapeutic approaches designed to destroy growth of malignant tumor cells are important for the treatment of lung cancer(12,13). The present study found that compound K could exhibit a therapeutic efficacy for lung cancer via HIF-1α-regulated glucose metabolism.

Panax Ginseng, a traditional medicinal herb, has been widely prescribed in oriental countries for thousands of years due to its diverse pharmaceutical effects, including anti-fatigue, anti-stress, anti-inflammation, adjusting blood pressure and immunoregulation (14,15). In addition, the anti-tumor effect of intake of ginseng has been reported in various types of human cancer (16). As a major ginsenoside metabolite, compound K has exhibited therapeutic potentials on human cancers (e.g., gastric cancer, hepatocellular carcinoma, lung cancer, leukemia) by regulating cancer cell growth, apoptosis, cell cycle arrest and metastasis (17,18). Consistently, our study also found that compound K inhibited the cell viability of lung cancer cells in a dose-dependent manner.

The "Warburg effects" refers to the phenomenon that cancer cells reprogram metabolic pathways for their bioenergetic and biosynthetic requirements. Increased glutamine metabolism, aerobic glycolysis, fatty acid and nucleotide synthesis has been linked to growth of malignant cancer cells (19). Correspondingly, inhibition of glucose metabolism may be a novel strategy against cancer progression. In our study, compound K treatment could inhibit the glucose uptake and lactate secretion and such differences were further increased under hypoxic conditions. It has been reported that many enzymes of glucose metabolism play a critical role in glucose metabolism, such as GLUTs, HK II, and LDHA (20). In our study, compound K administration obviously decreased the expression of HK II, PDK1 and LDHA both at mRNA and protein levels.

In the tumor microenvironment, cancer cells can adapt to low oxygen and switch on glycolytic metabolism to meet the demand for bioenergy (21). HIF-1α -induced glycolytic enzymes is required for energy production under the condition of insufficient oxygen supply (22). In addition, HIF-1α is considered to initiate the molecular response of oxygen-regulated genes under hypoxic conditions (23). Overexpression of HIF-1α has been reported in various human malignancies, such as breast, prostate, lung and liver cancers (24). Moreover, targeting of HIF-1α by chemical inhibitors may be a potential cancer therapy(25). In the present study, administration with compound K inhibited the expression of HIF-1α and its downstream gene, GLUT1, under normoxic and hypoxic conditions. To further validate the role of HIF-1α in the metabolic regulation, plasmids encoding HIF-1α were transfected into lung cancer cells. As a result, up-regulation of HIF-1α elevated glucose metabolism, contributing to enhanced lung cancer cell growth.

In conclusion, these data reveal that compound K may suppress the proliferation of NSCLC cells via HIF-1α mediated glucose metabolism. This study provides novel information of metabolic regulation of compound K, which contributes to its potential anticancer therapy by targeting glucose metabolism.

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