Original Article

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Radix Glehniae extract inhibits migration and invasion of lung cancer cells

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Abstract

Objective: The aim of this study was to investigate the effect of *Radix Glehniae* on the migration and invasion abilities of lung cancer cells.

Methods: Normal bronchial cell line 16HBE and lung cancer cell line SK-MES-1 were treated with *Radix Glehniae* extract. Proliferation, migration, and invasion abilities were determined by Cell Counting Kit (CCK)-8, Transwell, and Matrigel assays, respectively. The expression and secretion levels of tissue inhibitor of metalloproteinases 2 were detected by quantitative PCR and enzyme-linked immunosorbent assay, respectively.

Results: *Radix Glehniae* extract inhibited the migration and invasion abilities of SK-MES-1 cells and enhanced TIMP2 expression and secretion by SK-MES-1 cells, without causing toxicity to 16HBE cells.

Conclusion: *Radix Glehniae* is useful in lung cancer treatment.

Keywords: *Radix Glehniae*, lung cancer carcinoma, malignant behaviour

1 Introduction

Lung cancer is the most common cancer among men and the third most common cancer among women worldwide, with 1.59 million deaths every year [1]. The high mortality rate of lung cancer is mainly due to high rates of its invasion and metastasis. Surgery and radiotherapy cannot remove and kill metastatic tumor cells, and most of the currently used chemotherapy drugs have serious side effects that greatly reduce the therapeutic outcome and quality of life of patients [2]. Moreover, some chemotherapy drugs can induce normal tumor cells to become tumor stem cells, thus accelerating tumor invasion and metastasis [3]. It is therefore of utmost importance to develop nontoxic and efficient drugs to inhibit the invasion and metastasis of lung cancer cells.

Chinese medicine and pharmacy are a valuable source of knowledge that warrants in-depth exploration. These have accumulated extensive knowledge of tumor treatments over thousands of years of clinical practice. Besides killing tumor cells, Chinese medicine puts forward many other treatment ideas, such as "Fu Zheng Gu Ben", "clearing heat and detoxifying", "softening hardness and loosing knot", and so on. Some low-toxic and nontoxic Chinese herbs have played a role in tumor treatment [4]. *Radix Glehniae* is a commonly used nontoxic herb with the functions of "Yang yin qing fei", promoting the secretion of saliva or body fluid, and "expelling phlegm and arresting cough". It is useful for treating lung diseases and has been widely used in prescriptions against lung cancer [5]. Here, for the first time, we investigated the effect of *Radix Glehniae* on the migration and invasion abilities of lung cancer cells, as well as exploring the underlying mechanisms.

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2 Materials and instruments

2.1 Cells

Normal human bronchial epithelial cell line 16HBE and lung cancer cell line SK-MES-1 were purchased from the cell bank of Nanjing Kaiji Co. Ltd. (Nanjing, Jiangsu).

2.2 Drug

The crude drug of *Radix Glehniae* was purchased from the Inner Mongolia Hospital of Traditional Chinese Medicine (Huhhot, Inner Mongolia). The dried crude drug of *Radix Glehniae* was crushed into a powder and extracted using 75% ethanol two times, with each extraction lasting 40 minutes. The extracts were filtered and combined. The ethanol was recovered to obtain a thick paste, which was then freeze-dried to obtain the dry powder. The dry powder was dissolved in phosphate-buffered saline (PBS) to prepare a 100 mg/ml stock solution, which was sterilized using a 0.22-μm filter and stored at 4°C until use.

2.3 Reagents

Fetal bovine serum (FBS), RPMI-1640 medium, PBS, and trypsin were purchased from Gibco (Grand Island, NY, USA). Cell Counting Kit (CCK)-8 was provided by Amresco (Cleveland, Ohio, USA). Matrigel was purchased from BD Biosciences (San Diego, CA, USA). Total RNA extraction, reverse transcriptase, and fluorescence quantitative PCR (q-PCR) kits were all purchased from Takara (Dalian, Liaoning, China). Enzyme-linked immunosorbent assay (ELISA) kit was from Bai Zhi Co. Ltd (Beijing China).

2.4 Instruments

Carbon dioxide incubator (Thermo Fisher Scientific), super clean bench (Suzhou Jin Yan), inverted microscope (TE2000; Nikon), real-time PCR systems (7500; Applied Biosystems), and 8 μm Transwell device (Costar) were used.

2.5 Methods

2.5.1 Culture of cells

The bronchial epithelial cell line 16 HBE and SK-MES-1 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 0.1% penicillin-and-streptomycin (complete RPMI-1640 medium) at 37°C in a humidified atmosphere containing 5% CO_2. The cells in logarithmic growth phase were used.

2.5.2 CCK-8 assay

The viability of 16 HBE cells was measured using CCK-8 analysis. The 16 HBE cells were suspended in complete RPMI-1640 medium at a concentration of 2.5×10^4 cells/ml, and 0.2 ml of the suspension was added into each well of 96-well plates. After 12 hour culture, the cells were treated with different concentrations of *Radix Glehniae* extract (5 mg/ml, 10 mg/ml, and 15 mg/ml) or PBS. After treatment for 24 hours, 20 μl CCK-8 solution was added into each well. Then, the plates were continuously incubated for 2 hours

in a humidified CO_2 incubator at 37°C. Finally, the absorbance of the sample taken from each well was measured using a microplate reader at 450 nm.

2.5.3 Determination of migration capacity

SK-MES-1 cells were serum-starved for 24 hours and suspended in serum-free RPMI-1640 medium (10^6/ml). Every 100 µl of the cell suspension was seeded into each upper chamber, and 600 µl of RPMI-1640 containing 20% FBS was added into the lower chamber. The *Radix Glehniae* extracts were added into the upper chambers. The plates were incubated for 24 hours at 37°C, the media were removed from the Transwell chambers, and the cells on the upper surface of the Transwell membrane were wiped off. Cells that had migrated to the lower surface of the Transwell membrane were fixed and stained with crystal violet, and the number of cells in five randomly selected fields at ×200 magnification was counted.

2.5.4 Determination of invasion capacity

Matrigel was diluted with a pre-cooled RPMI-1640 medium. The upper surfaces of the Transwell membranes were precoated with 100 µl diluted Matrigel, which was allowed to solidify at 37°C for 4 hours. SK-MES-1 cells were serum-starved for 24 hours and suspended in RPMI-1640 medium (10^6/ml). Every 150 µl of the cell suspension was seeded into each upper chamber, and 600 µl of RPMI-1640 containing 20% FBS was added into the lower chamber. The *Radix Glehniae* extract was added into the upper chambers. The plates were incubated for 24 hours at 37°C, the media were removed from the Transwell chambers, and the cells on the upper surface of the Transwell membrane were wiped off. Cells that had migrated to the lower surface of the Transwell membrane were fixed and stained with crystal violet, and the number of cells in five randomly selected fields at ×200 magnification was counted.

2.5.5 Detection of mRNA expression

SK-MES-1 cells after passage for 24 hours were treated with different concentrations of *Radix Glehniae* extract (5 mg/ml, 10 mg/ml, and 15 mg/ml) or PBS for 24 hours. Then, the cells were collected, and total RNA was isolated and reverse-transcribed. Q-PCR was performed, with the cycling conditions as follows: 95°C for 30 seconds, and 40 cycles of 95°C for 5 seconds and 60°C for 31 seconds. Relative expression levels of each small RNA were calculated with the $2^{-\Delta\Delta Ct}$ method using glyceraldehyde 3-phosphate dehydrogenase as endogenous control.

2.5.6 ELISA test

SK-MES-1 cells were treated with different concentrations of *Radix Glehniae* extract (5 mg/ml, 10 mg/ml, and 15 mg/ml) or PBS for 12 hours. Then, the culture medium was replaced with serum-free RPMI-1640 medium. After 24 hours, the tissue inhibitor of metalloproteinases 2 (TIMP2) concentration of the medium was measured using an ELISA Kit according to the manufacturer's instructions.

2.6 Statistical analysis

Statistical analysis was performed with the SPSS19.0 software. One-way analysis of variance was used to analyze the data, and the significance level was set at $P<0.05$.

3 Results

3.1 *Radix Glehniae* extract caused no toxicity to 16 HBE cells

The CCK-8 results showed that the 16 HBE cells treated with 1 mg/ml, 5 mg/ml, 10 mg/ml, and 15 mg/ml extracts displayed similar viability as the control-treated 16 HBE cells (Figure 1), indicating that the *Radix Glehniae* extract has no toxicity to 16 HBE cells.

3.2 *Radix Glehniae* extract inhibited the migration and invasion abilities of SK-MES-1 cells

The Transwell and Matrigel assays showed that 5 mg/ml, 10 mg/ml, and 15 mg/ml extracts inhibited the migration and invasion abilities of SK-MES-1 cells, respectively. The inhibitory effect increased with the increase in extract concentration (Table 1).

3.3 *Radix Glehniae* extract upregulated the expression of TIMP2 mRNA in SK-MES-1 cells

Q-PCR results showed that 5 mg/ml, 10 mg/ml, and 15 mg/ml extracts upregulated the TIMP2 mRNA expression in SK-MES-1 cells. The upregulation increased with the increase in extract concentration (Table 2).

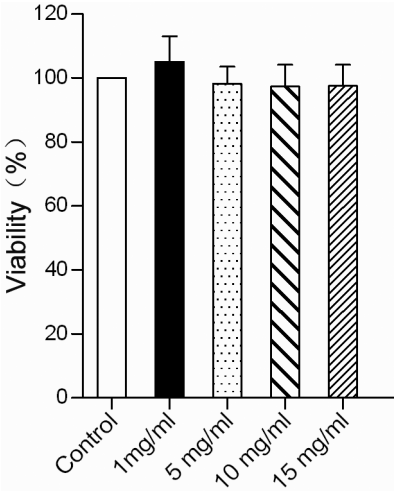

Fig. 1: The effect of *Radix Glehniae* extract on the viability of 16HBE cells.

Tab. 1: Effects of *Radix Glehniae* extract on the migration and invasion abilities of SK-MES-1 cells

Groups	Migrated cells	Invasive cells
Control	127±8	66±6
Extract concentration		
5 mg/ml	107±9*	49±3**
10 mg/ml	77±8**	36±3**
15 mg/ml	51±6**	23±6**

*P<0.05 vs. control group, **P<0.01 vs. control group.

Tab. 2: The effect of *Radix Glehniae* extract on TIMP2 mRNA expression in SK-MES-1 cells

Groups	Relative mRNA expression
Control	1.00±0.00
Extract concentration	
5 mg/ml	1.33±0.07**
10 mg/ml	1.55±0.07**
15 mg/ml	1.94±0.05**

**$P<0.01$ vs. control group.

Tab. 3: The effect of *Radix Glehniae* extract on TIMP2 mRNA secretion by SK-MES-1 cells

Groups	TIMP2 concentration (pg/ml)
Control	76.2±4.4
Extract concentration	
5 mg/ml	100.6±10.4**
10 mg/ml	131.3±2.8**
15 mg/ml	163.1±8.9**

**$P<0.01$ vs. control group.

3.4 *Radix Glehniae* extracts enhanced TIMP2 secretion by SK-MES-1 cells

ELISA results showed that 5 mg/ml, 10 mg/ml, and 15 mg/ml extract treatment increased the TIMP2 concentration in the culture medium of SK-MES-1 cells, indicating that *Radix Glehniae* extract enhanced TIMP2 secretion by SK-MES-1 cells (Table 3).

4 Discussion

The migration and invasion of cancer cells are important causes for the death of cancer patients. Most of the existing drugs that inhibit the migration and invasion of cancer cells have strong toxic side effects on patients, causing serious injuries to the liver, kidneys, and the bone marrow, which reduces their overall clinical efficacy [6]. *Radix Glehniae* is a commonly used nontoxic traditional Chinese medicine and has been used in many anticancer prescriptions. However, the exact anticancer mechanism of *Radix Glehniae* has not been fully investigated. The current study shows that *Radix Glehniae* has no cytotoxic effect on human normal bronchial epithelial cells, and it can effectively inhibit the migration and invasion abilities of lung cancer cells.

TIMP2 is an important member of the tissue inhibitor of metalloproteinases family and is a strong tumor-suppressive factor [7]. It can bind to the matrix metalloproteinases in extracellular matrix and block their active sites that catalyze the degradation of extracellular matrix, thus, preventing these enzymes from promoting cancer cell migration and invasion [8]. Lung cancer cells usually synthesize and secrete a lower level of TIMP2 than normal cells. Here, we show that *Radix Glehniae* extract treatment enhanced the synthesis and secretion of TIMP2 by lung cancer cells, providing an important explanation to its antimigration and anti-invasion mechanism.

In other words, we have investigated the anticancer mechanism of *Radix Glehniae* at the cellular and molecular levels, the results of which provide a theoretical basis for the clinical application of *Radix Glehniae* in cancer treatment. Our results also demonstrate that developing new anticancer drugs from traditional Chinese herbs, especially nontoxic Chinese herbs, is a promising direction to follow.

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