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RESEARCH ARTICLE

Effect of compound Guizhu capsule on phosphate and tension homology deleted on chromsome ten and murine double mimute 2 gene expression in lung cancer of mice

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Abstract

OBJECTIVE: To observe the inhibitory effect of the Chinese herbal compound Guizhu capsule (CGZC) on lung cancer and explore the possible mechanism underlying its actions by evaluating its effects on the expression of phosphate and tension homology deleted on chromsome ten (PTEN) and murine double mimute 2 (MDM2) in lung cancer model mice.

METHODS: A mouse model of transplanted lung cancer was established by subcutaneous inoculation of cancer cells into the axillae of mice. Twenty-four hours later, the mice were weighed and randomly divided into the model control group, cisplatin group (DDP group), and high, moderate and low dosage CGZC groups, with ten mice in each group. The control mice received an equal volume of distilled water. DDP was intraperitoneally injected at 1 mg/kg in the DDP group, once a day for 3 days. CGZC diluted with distilled water was administered at 20 g/kg (10 times the clinical adult dosage) in the high-dose group, 10 g/kg (5 times the clinical adult dosage) in the moderate-dose group and 5 g/kg (2.5 times the clinical adult dosage) in the low-dose group once a day for 10 days. On the 11th day, the mice were weighed and killed. The tumor tissues were weighed and the tumor inhibition rate was calculated. PTEN and MDM2 protein expression were detected by immunohistochemical analysis of tumor tissues.

RESULTS: The CGZC high- and moderate-dose groups showed a significant inhibitory effect on tumor growth ($P < 0.05$); there was no significant difference between the high dose group and the DDP group ($P > 0.05$). The CGZC high-dose group also showed enhanced expression of PTEN protein ($P < 0.01$) and decreased expression of MDM2 protein ($P < 0.01$) in lung cancer cells of the mice. There was no significant difference between the high dose group and the DDP group.

CONCLUSION: CGZC has a significant inhibitory effect on transplanted lung cancer in mice. The mechanism may involve reducing expression of PTEN and decreasing the expression of MDM2 in the lung cancer tissues of the mice.

Keywords: Lung neoplasms; Transplantion; Immunohistochemistry; PTEN phosphohydrolase; Proto-oncogene proteins c-mdm2; Ubiquitin-protein ligases; Compound Guizhu capsule

INTRODUCTION

Lung cancer shows high clinical morbidity and mortality rates. Thus, how to effectively treat lung cancer has remained a hot topic. Studies have demonstrated murine double mimute 2 (MDM2) mouse double minute gene is a recently discovered apoptosis inhibitory protein gene and is also the strongest one which has been found in most human tumor tissues. Phosphate and tension homology deleted on chromsome ten (PTEN) is a tumor suppressor gene, whose protein expression is related to some malignancies, development, biol ogical behavior and prognosis. PTEN gene mutation exists in many tumors, deletions, methylation and other genetic changes, PTEN protein expression with some malignancies, development, biological behavior and prognosis.[1]

The project based on years of clinical experience and Traditional Chinese Medicine (TCM), according to the theory of TCM proposed that lung and kidney *Qi* deficiency is the internal factor of lung cancer, toxin invasion is the incidence of external conditions, stagnation of *Qi* and blood stasis, and toxin stasis have been established as an important pathological basis for the formation of lung cancer. The compound Guizhu capsule (CGZC) consists of astragalus root, cinnamon, Fructus Psoraleae and zedoary, with reported effects in nourishing the lung, removing blood stasis and detoxification. Clinical observations showed that the CGZC treatment in patients with lung cancer can improve the clinical symptoms, reduce the toxic side effects of chemotherapy, improve quality of life, reduce recurrence and metastasis, and prolong survival during the course of advanced lung cancer, chemotherapy for lung cancer and rehabilitation after chemotherapy.

In this study, we investigated the effect of CGZC on lung cancer and the potential mechanism. Lung cancer models in mice were established by subcutaneous inoculation of cancer cells into axillae of mice. We evaluated the effects of CGZC treatment on tumor rate and MDM2 and PTEN expression levels.

MATERIALS AND METHODS

Drugs

CGZC was provided by the traditional Chinese drug hospital preparation room of the College of Pharmacy of Jinan University. CGZC is composed of Huangqi (*Radix Astragali Mongolici*) 75 g, Rougui (*Cortex Cinnamomi Cassiae*) 50 g, Buguzhi (*Fructus Psoraleae*) 50 g, Ezhu (*Rhizoma Curcumae Phaeocaulis*) 75 g, Xianhecao (*Herba Agrimoniae*) 50 g, Banzhilian (*Herba Scutellariae Barbatae*) 50 g, and Gualoupi (*Percarpium Trichosanthis Kirlowiis*) 50 g. Each tablets 0.5 g, equivalent to 2 g's originated herbs, batch number, 091014. Equivalent to the original drug 2 g.

Reagents

Rabbit anti-mouse PTEN and rabbit anti-mouse MDM2 antibodies were obtained from Wuhan Boster. The SABC (strept avidin-biotin complex) kit and DAB (Diaminobenzidine) color reagent box (AR1022) were purchased from Wuhan Boster. EDTA antigen repairing liquid (pH 8.0), antibody dilutions were from Beijing Zhongshan Golden Bridge biotechnology company.

Animals

C57BL/6J mice of Specific pathogen free grade (6-8-weeks-old, with body weight 18-22 g, equal numbers of males and females) were purchased from the Medical Experimental Animals Center of Zhongshan University (Certificate No. SCXK 2009-0011).

Tumor cells

Lewis lung cancer cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences.

Instruments

The following instruments were used in this study: AS-325 slicer (Leica, Germany); TK-218V type constant temperature baking sheet machine (Hubei Taiwei Medical Technology Co., Ltd.); TB-718 biological tissue package embedding machine (Hubei Taiwei Medical Technology Co., Ltd.); TC-120S program-controlled intelligent biological tissue automatic dehydration machine (Hubei Taiwei Medical Technology Co., Ltd.); and purification worktable (Hangzhou Purification Equipment Co., Ltd.).

Lung cancer mouse model and treatment groups

Under aseptic condition, take passaged 10 days of Lewis lung cancer tumor mice, killed by avulsion cervical spine, aseptic operation from axillary subcutaneous

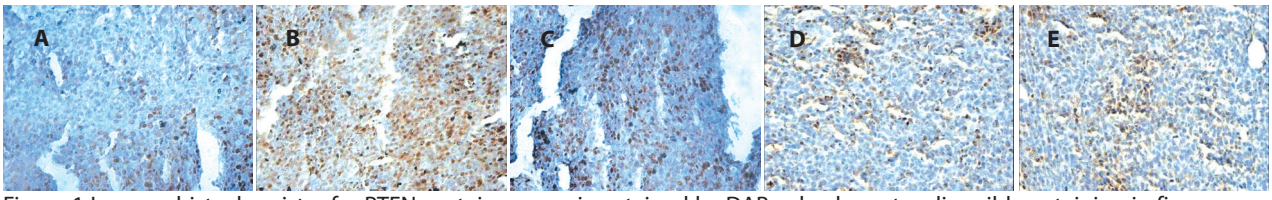

Figure 1 Immunohistochemistry for PTEN protein expression stained by DAB color, hematoxylin mild re-staining in five groups (× 200)

A: model group; B: cisplatin (DDP) group; C: compound Guizhu capsule (CGZC) high-dose group; D: CGZC moderate-dose group; E: CGZC low-dose group. Model group received an equal volume of distilled water, 10 days; DDP group intraperitoneally injected DDP, 1 mg/kg, once a day, for 3 days; CGZC high-dose group was administered CGZC, 20 g/kg, once a day, 10 days; CGZC moderate-dose group was administered CGZC, 10 g/kg, once a day, 10 days; CGZC low-dose group was administered CGZC, 2.5 g/kg, once a day, 10 days. PTEN: phosphate and tension homology deleted on chromsome ten.