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EXPERIMENTAL STUDY

Enhancement of radiosensitivity of lung adenocarcinoma using a decoction from the Fuzhengzengxiao formula

Zhang Qiaoli, Wu Tong, Lei Yong , Li Bo, Liu Weiyi, Tian Yehong, Zhao Weipeng, Huang Jingchang

Zhang Qiaoli, Department of Traditional Chinese Medicine, Beijing Hepingli Hospital, Beijing 100013, China
Wu Tong, Department of Traditional Chinese Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, 100015, China
Lei Yong, Liu Weiyi, Tian Yehong, Zhao Weipeng, Graduate School of Beijing University of Traditional Chinese Medicine, Beijing 100029, China
Li Bo, Health Science Center, Peking University, Beijing 100191, China
Huang Jingchang, Department of Acupuncture and Minimally Invasive Oncology, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, 100029, China
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Correspondence to: Prof. Huang Jingchang, Department of Acupuncture and Minimally Invasive Oncology, Beijing University of Chinese Medicine Third Affiliated Hospital, Beijing, 100029, China zryhhuang@163. com
Telephone: +86-10-84205733
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Abstract

OBJECTIVE: To study the effects of a decoction of Fuzhengzengxiao formula on lung adenocarcinoma regarding the inflammatory protein S100A9 known to enhance cancer cell sensitivity.

METHODS: A nude mouse model of human lung adenocarcinoma was established. The mice were randomly divided into four groups using the random number table method: Group Ⅰ, control; Group Ⅱ, treatment with a decoction of the Fuzhengzengxiao formula alone; Group Ⅲ, treatment with radiotherapy alone; and Group Ⅳ, treatment with radiotherapy plus a decoction of Fuzhengzengxiao formula. When the tumor body was 1 cm^3 in diameter, the tumor bearing mice in Groups Ⅲ and Ⅳ were irradiated at a single dose of 10 Gy and the tumor inhibition rate was evaluated. The expression of S100A9 was determined using Western blotting and q-PCR (Real-time Quantitative PCR Detecting System). The sensitivity of cells containing RNAi S100A9 to radiotherapy was evaluated using the Click multiple target model,and the cell cycle was analyzed using flow cytometry.

RESULTS: Relative to the control group,the expression of S100A9 in the tumors in each treatment group was decreased,especially in Group Ⅳ. The sensitizing enhancement ratio (SER) Dq was >1 after RNAi S100A9; it decreased the surviving fraction after a 2 Gy dose exposure,and also the D_0 and Dq of the tumor cells; in addition, the radiosensitivity of G_2/M cells was significantly increased.

CONCLUSION: The decoction of the Fuzhengzengxiao formula downregulated the expression of S100A9 in lung adenocarcinoma cells.

Key words: Calgranulin B; RNA interference; Radiation tolerance; Adenocarcinoma of lung; Fuzhengzengxiao formula

INTRODUCTION

Lung cancer is one of the most fatal tumors worldwide. Eighty percent of non-small cell lung cancer patients

have a metastasis rate of ≤ 70%; because of aging and other associated diseases, most patients cannot tolerate surgical treatment and consequently radiotherapy is the main treatment method. However, the treatment failure rate ranges from 60% to 70%.[1-3] Previous studies have reported that the Fuzhengzengxiao formula significantly improved the efficacy and reduced the side effects of radiotherapy in lung cancer patients; however, the pathogenesis regarding its enhancement of the efficacy of radiotherapy remains unclear. It has been found that in mice that were treated with the Fuzhengzengxiao formula before irradiation, S100A9 expression in tumor cells was obviously abnormal (when assayed using protein microarray technology), relative to mice that received treatment with radiation alone. We hypothesized that S100A9 may be involved in enhancing tumor radiosensitivity after treatment with the Fuzhengzengxiao formula. The objective of the present study was to investigate how S100A9 influenced cancer cell sensitivity to radiotherapy while the cancer was being treated with a decoction of the Fuzhengzengxiao formula.

MATERIALS AND METHODS

Drugs and reagents

The Fuzhengzengxiao formula is composed of Huangqi (*Radix Astragali Mongolici*), Shihu (*Herba Dendrobii Nobilis*), Beishashen (*Radix Glehniae*), Jinyinhua (*Flos Lonicerae*), Honghua (*Flos Carthami*), Sumu (*Lignum sappan*), Baizhu (*Rhizoma Atractylodis Macrocephalae*), Taizishen (*Radix Pseudostellariae*), Gouqizi (*Fructus Lycii*), Jixueteng (*Caulis Spatholobi*), Fuling (*Poria*) and Jineijin (*Endothelium Coreneum Gigeriae Galli*), which were decocted twice. The decoctions were mixed and concentrated into 2.2 g of crude drugs/mL, and then disinfected by boiling. RNAi S100A9 was provided by Cyagen Biosciences Inc., (Guangzhou, China); it was subcultured in RPMI1640 containing 10% fetal calf serum. Calf serum and diethyl pyrocarbonate (DEPC) were purchased from the Sigma Company (St. Louis, Missouri, USA). Rabbit anti-S100A9 antibody was purchased from the Proterintech Company (Chicago, IL, USA).

Animals and the PAa cell line

The experimental animals were 6-week-old male BALB/C-nu/nu nude mice. They were purchased from the Institute of Experimental Animals, Chinese Academy of Medical Sciences, and raised in specified pathogen free conditions (certificate of quality No. SCXKJ 2009-004). The low transfer human pulmonary adenocarcinoma was purchased from the Pathological Section of the Medical Department, Peking University (Beijing, China).

Establishment of animal models

Pulmonary adenocarcinoma cells in the logarithmic growth phase were taken. They were prepared at a concentration of 5×10^6 cells/mL with RPMI1640 culture medium; 0. 3 mL of these cells was injected into the armpit of the forelimb of each nude mouse. The mouse were randomly divided into four groups (each group have 28 nude mouse)using the random number table method: Group Ⅰ, control; Group Ⅱ, treated with the decoction of the Fuzhengzengxiao formula alone; Group Ⅲ, treated with radiotherapy alone; and Group Ⅳ, treated with radiotherapy plus the decoction of the Fuzhengzengxiao formula. From the day of tumor inoculation, each nude mouse in Groups Ⅱ and Ⅳ was intragastrically administered 0.5 mL (1.1 g crude drug) of the decoction of the Fuzhengzengxiao formula daily, and the other two groups were administered an equal volume of distilled water. When the tumor had reached a size of 1 cm^3 (at about 28 days after inoculation) the mouse in Groups Ⅲ and Ⅳ were irradiated at a single dose of 10 Gy. In 18 nude mouse from each group, the tumors were removed and homogenized at 6, 12 and 24 h after irradiation. Changes in the tumor growth rate were monitored in the 10 remaining mouse in each group.

Tumor inhibition rate

To observe changes in tumor volume, this was measured 1 day before radiotherapy and every 2 days after radiotherapy for a total of 10 times; mouse were killed after the last measurement, and the weight of the tumor was measured and the growth inhibition rate calculated. The tumor inhibition rate = (C – T)/C × 100%, where C is the mean weight of the tumors in the negative control group, and T is the mean weight of the tumors in a given experimental group.

Detection of S100A9 protein expression

First, 200 μL of protein lysate was added into a centrifuge tube holding 20 mg of tumor, which was homogenated and placed on ice for 15 min, and centrifuged at 4 ℃ (9000 ×g for 10 min); the supernatant was draw to determine the protein content. Protein loads were 20 μg/lane. It was separated using 12% separation gel and 5% stacking gel. Then the protein was semi dryly transferred to PVDF film, 8-mL anti-S100A9 was diluted using closed liquid (S100A9, 1∶500 dilution; actin, 1∶1500 dilution) and reacted at 4 ℃ overnight; the second antibody (1∶10000) was reacted for 4 h. The ECL chemiluminescence method, exposure to X-rays and scanning film JX330 (Sharp Electronics, Tokyo, Japan) involving a transmission scanner were used for analysis. Finally, LabWorks software was used to evaluate the image grayscale.

Detection of the mRNA expression of S100A9

Extraction of total RNA and first strand synthesis of cDNA were carried out according to the instructions of the manufacturer. Real time PCR was performed in a 25 μL reaction system. It included: 10 μL of 2 ×