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Original Article

Glycyrrhizin treatment inhibits proliferation and invasive potential of lung cancer cells

Yong-Fu Ma*, Nan-Nan Guo*, Jian Chu, Shuai Jin, Bo Yang, Jie Li, Tao Zhang, Jun-Tang Guo, Lei Chen, Chao-Yan Liang, Ling-Hui Wang, Yang Liu

*Department of Thoracic Surgery, Chinese PLA General Hospital, Beijing 100853, China. *Equal contributors.*

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Abstract: The present study was aimed to investigate the effect of glycyrrhizin onosteopontin (OPN) expression, cell proliferation, invasion potential and cell cycle distribution inhuman lung carcinoma cell line, HCC827. The results revealed a significant inhibition in the expression of OPN protein on treatment with 100 μM doses of glycyrrhizin after 48 h in HCC827 cells. Glycyrrhizin treatment also inhibited the rate of cell proliferation and caused cell cycle arrest by preventing the cells from entering into the G2 phase. Exposure of HCC827 cells to glycyrrhizin at 100 μM concentration inhibited the invasion potential significantly compared to the control cells. In addition, glycyrrhizin treatment reduced the expression of MMP-2 and MMP-9 significantly after 48 h inHCC827 cells. Thus, glycyrrhizin treatment inhibits proliferation, invasion potential and arrests cell cycle in lung cancer cells through down-regulation of OPN expression. Therefore, glycyrrhizin can be of therapeutic importance for the treatment of lung cancer.

Keywords: Glycyrrhizin, invasion potential, osteopontin, therapeutic, cell cycle

Introduction

Lung cancer is one of the main causes of cancer related deaths and has very high rate of incidence in the developed countries [1]. In United States alone more 160,000 deaths occur because of lung cancer every year [1]. It has been observed that 5-year survival rate for lung cancer patients with stage IIa and stage IIIb is 9-25% and 3-7%, respectively. Despite advancement in the field of cancer treatment by radical resection and chemotherapy the rate of recurrences in lung cancer patients is very high [2, 3]. Many combination therapies were also developed for the treatment of lung cancer patients but the results obtained were unsatisfactory [4]. Therefore, attempts for the development of new strategies for lung cancer treatment are being constantly performed.

Plant based natural products have been investigated for the treatment of various disorders including, cancer, neurodegerative diseases, etc. The extract of the root (licorice) of *Glycyrrhizaglabra* has been used for the treatment of several disorders in oriental herbal medicine. In Chinese traditional medicine licorice has been used for detoxification and cure of swelling [5]. Licorice forms the component of drugs and beverages as a sweetening agent [6]. The phytochemical investigation of the licorice has led to the isolation of glycyrrhizin [7]. Pharmacological analysis of the glycyrrhizin has revealed its promising anti-tumorigenic [8], immunomodulatory [9], anti-inflammatory [10] and anti-ulcer [11] activities. In addition, antiviral activity of the glycyrrhizin against hepatitis B virus, HIV has also been reported [12]. In the present study effect of glycyrrhizin on OPN expression, cell proliferation, invasion potential and cell cycle distribution inhuman lung carcinoma cell line, HCC827 was investigated.

Materials and methods

Cell culture

The human lung carcinoma cell line, HCC827 was purchased from the Shanghai Institute of Biochemistry and Cellular Biology Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8-10% fetal bovine serum in humidified atmosphere of CO_2 at 37°C.

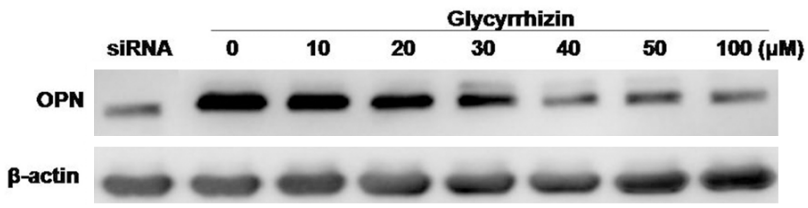

Figure 1. Glycyrrhizin exhibits concentration dependent inhibitory effect on the expression of OPN protein in HCC827 cells. The effect of OPN silencer RNA on expression of OPN protein was similar to that of 100 μM concentration of glycyrrhizin. The expression of β-actin was taken as the control.

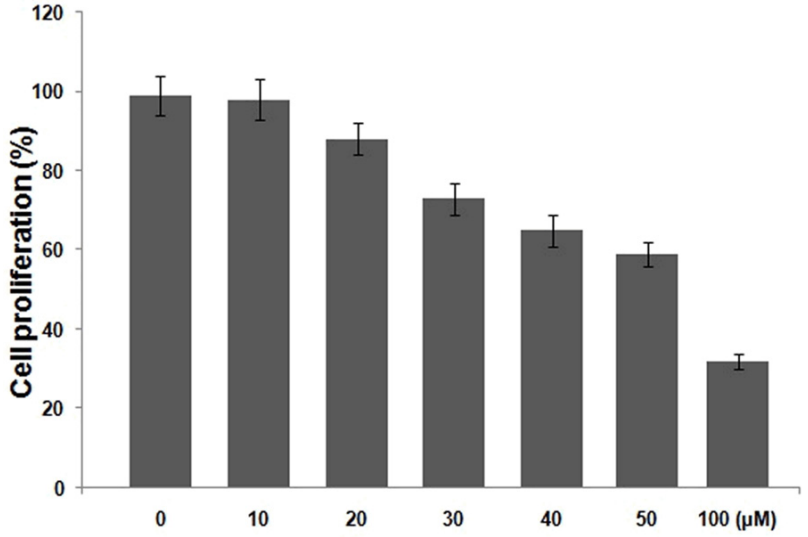

Figure 2. Glycyrrhizin treatment inhibits proliferation of analysis HCC827 cells. The cells were distributed in 96-well plates and treated with glycyrrhizin for 48 h followed by MTT assay. Values represent the means ± SD of 3 wells.

Materials

Glycyrrhizinand dimethyl sulphoxide (DMSO) were purchased from the Sigma-Aldrich (SaintLouis, MO, USA). Dulbecco's modified Eagle's medium was obtained from Gibico (BRL, Guangzhou, China). OPN silencer vector was constructed using the previously reported protocol [13].

Proliferation assay

Proliferation in human lung carcinoma cell line, HCC827 was determined by using the MTT assay. The cells were distributed at the density of 2.5 × 10^5 cells per ml onto 96-well plate. Following 48 h of culture, the cells were incubated with 10 to 100 μM concentration of glycyrrhizin for 48 h. After incubation, 50 μL MTT (5 mg/mL) solution was added to each of the well and incubation was continued again for 4 h more. To each well 150 μL of DMSO was added for dissolving the formazon crystals formed. The measurement of absorbance was performed at 465 nm three times and cell survival rate was determined.

Migration assay

Migration potential of HCC827 cells was determined using Transwell (Costar) system. For this purpose, the cells at a density of 2 × 10^6 cells per ml were treated with glycyrrhizin for 48 h, trypsinized, washed and then resuspended in DMEM. Into the bottom compartment of the well DMEM (500 μL) supplemented with 10% FCS was placed whereas the upper compartments was filled with 150 μL of cell suspension in DMEM devoid of FCS. Following 24 h of cultured in a humidified atmosphere of 5% CO_2 at 37°C the membrane was washed with PBS. The cells were fixed inmethyl alcohol for 20 min followed by crystal violet staining. The cells remaining in the upper surface of the membrane were cleaned using cotton. The high-power Olympus-CX31 microscope (Olympus Corp., Tokyo, Japan) was used for the calculation of the cells present on the lower surface of the membrane. The counting of the cells was performed in triplicates.

Flow cytometry assay

The effect of glycyrrhizin on cell cycle distribution in HCC827 cells was analyzed using flow cytometry analysis of the DNA. CycleTEST™ PLUS DNA reagent kit (BD Biosciences Pharmingen, USA) was used for the labelling of DNA. The analysis of the DNA samples was then performed by the application of flow cytometer (Beckman Coulter, Brea, CA, USA).

Western blot analysis

HCC827 cells after treatment with glycyrrhizin for 48 h were suspended at a density of 2 × 10^6

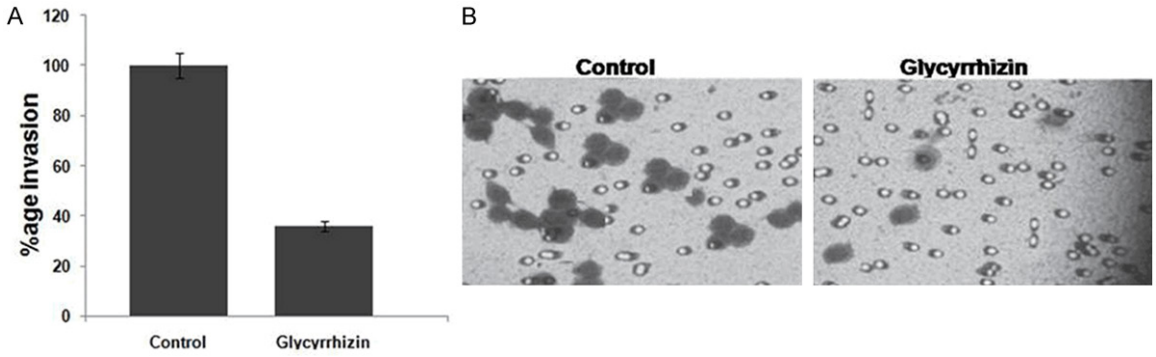

Figure 3. Glycyrrhizin treatment inhibits the migration potential of HCC827 cells. The HCC827 cells were treated with 100 μM concentration of glycyrrhizin and the analyzed using by Transwell assay. The data expressed are the means ± SD of relative migration vs. the control from 3 independent experiments.

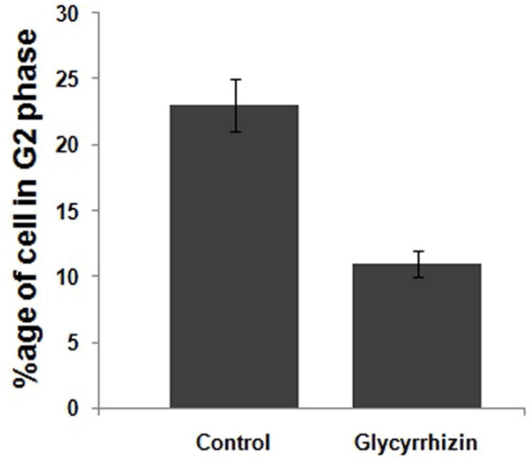

Figure 4. Flow cytometry analysis of cell cycle in HCC827 cells. The cells were treated with glycyrrhizin (100 μM) and then analyzed by flow cytometry. The data expressed are the means ± SD of three experiments performed independent.

cells in 200 μL lysis buffer (40 mmol/L Tris-HCl, 150 mmol/LKCl, 1 mmol/L EDTA, 100 mmol/L $NaVO_3$, 1% Triton X-100 and 1 mmol/L PMSF, pH 7.5). The NucBuster™ Protein Extraction kit (Novagen®; Merck KGaA, Darmstadt, Germany) was used for harvesting the cell lysates according to the manual protocol. Equal volumes of the proteins were resolved on 10% SDS-PAGE followed by transfer to polyvinylidene fluoride membranes (Millipore Corp., Billerica, MA, USA). The non-specific sites in the membranes were blocked using non-fat milk [5% in Tris-buffered saline with Tween®-20 (TBST) buffer]. Incubation of the membranes with primary antibodies for OPN (Sigma, USA), β-actin (PTG, USA), MMP-2 and MMP-9 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed at 4°C for overnight. The membranes were then washed with PBS followed by incubation with horseradish peroxidase-conjugated goat anti-mouse (EK010) or anti-rabbit (EK020) immunoglobulin G (Zhuangzhi Bio, Xi'an, China) at room temperature for 1 h. Enhanced Chemiluminescence kit (ECL Plus; GE Healthcare Europe GmbH, Freiburg, Germany) was used for the visualization of the bands.

Statistical analysis

For the processing of obtained data Statistical Package for Social Sciences (SPSS for Windows, version 17.0; SPSS, Inc., Chicago, IL, USA) was used. The monofactorial analysis of variance was used for analysis. The data expressed are the mean ± standard deviation. The statistically significant differences were considered at a *P*-value of <0.05.

Results

Effect of glycyrrhizinon expression of OPN in HCC827 cells

The results from western blot analysis revealed that glycyrrhizin treatment caused a significant reduction in the expression of OPN protein. The reduction in OPN protein expression in HCC827 cells by glycyrrhizin was found to be concentration dependent. Among the range of glycyrrhizin concentrations from 10 to 100 μM tested the reduction in OPN expression was significant at 100 μM after 48 h compared to the control cells (**Figure 1**). To confirm the reduction in OPN expression, HCC827 cells were transfected with OPN silencer RNA. The results revealed that transfection of OPN silencer RNA resulted

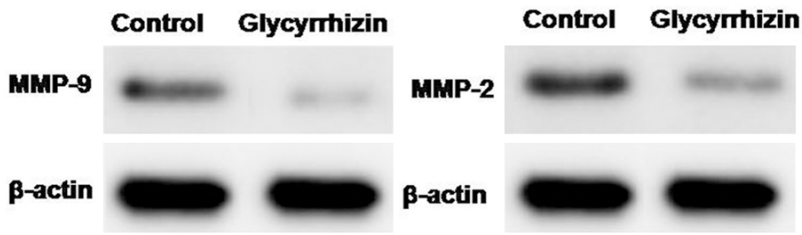

Figure 5. Glycyrrhizin treatment inhibited the expression ofMMP-2 and MMP-9 in HCC827 cells. The cells were incubated with 100 μM concentration of glycyrrhizin and then subjected to western blot analysis.

similar effect on the OPN expression as that of glycyrrhizin.

Effect of glycyrrhizinon proliferation of HCC827 cell

Glycyrrhizin treatment exhibited a significant inhibitory effect on the rate of proliferation in HCC827 cells. The inhibition in HCC827 cell proliferation was significant at 100 μM concentration after 48 h (**Figure 2**).

Effect of glycyrrhizinon migration potential of HCC827 cells

Transwell assay was used to analyze the effect of glycyrrhizinon the migration potential of HCC827 cells. It was observed that exposure of HCC827 cells to glycyrrhizin resulted in a significant reduction in the migration potential. Compared to the control cells the migration potential of HCC827 cells were significantly inhibited at 100 μM concentration following 3 days of the treatment (**Figure 3**).

Effect of glycyrrhizinon cell cycle distribution

The results from flow cytometry demonstrated that glycyrrhizin treatment for 48 h caused a significant reduction in the population of HCC827 cells in G2 phase (**Figure 4**). Thus glycyrrhizin treatment at 100 μM concentration inhibited proliferation of HCC827 cells by preventing the cells from entering into G2 phase of cell cycle.

Effect of glycyrrhizin on the expressions of MMP-2 and MMP-9

We also examined the effect of glycyrrhizin on the expression of MMP-2 and MMP-9 in HCC827 cells. The results revealed that glycyrrhizin treatment caused a marked reduction in the expression of MMP-2 and MMP-9. The reduction was significant after 48 h at 100 μM concentration of glycyrrhizin compared to the control cells (P=0.001, **Figure 5**).

Discussion

The present study was aimed to investigate the effect of glycyrrhizin on proliferation, migration potential, cell cycle distribution and the underlying mechanism in human lung carcinoma cells. The results revealed that glycyrrhizin treatment inhibited the rate of proliferation, suppressed migration potential and arrested cell cycle by preventing the cells to enter G2 phase. All these effects were found to be induced through the inhibition of OPN expression.

In various types of cancers osteopontin expression is up-regulated and exhibits an important role in the cell proliferation, migratory and invasive potential [14]. Enhanced expression of OPN in the carcinoma cells is also responsible for resistance to various chemotherapeutic agents [15]. Results from the present study showed that exposure of HCC827 cells to glycyrrhizin significantly inhibited the expression of OPN in a concentration dependent manner. Since OPN is involved in the proliferation of carcinoma cells analysis of the cell proliferation was also performed. The results revealed a significant inhibition in the rate of HCC827 cell proliferation on treatment with glycyrrhizin. Analysis of the migration potential using Transwell assay showed a significant reduction in the invasive potential of HCC827 cells on exposure to glycyrrhizin. It is reported that OPN also plays a vital role in the distribution of cells in various phases of the cell cycle [16]. The results from the present study revealed that treatment of HCC827 cells with glycyrrhizin arrested cell cycle by preventing the cells from entering into G2 phase.

Other factors reported to play an important role in the proliferation and invasion of the carcinoma cells include MMP-2 and MMP-9 [17]. The results from the present study showed that glycyrrhizin treatment inhibited the MMP-2 and MMP-9 expression in the human lung cancer cells.

Thus, glycyrrhizin treatment inhibits proliferation, invasion potential and arrests cell cycle in lung cancer cells through down-regulation of OPN expression. Therefore, glycyrrhizin can be of therapeutic importance for the treatment of lung cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Yang Liu and Ling-Hui Wang, Department of Thoracic Surgery, Chinese PLA General Hospital, 28 Fuxing Road, Beijing 100853, P. R. China. Tel: 0086-10-66938013; Fax: 0086-010-66938013; E-mail: liuyangda@hotmail.com

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