Original Article
Role of SB203580 in the regulation of human esophageal cancer cells under the effect of Diosgenin

Weiliang Ding¹*, Yancai Jiang²*, Yaping Jiang³*, Taofeng Zhu⁴, Ying Xu⁵, Wenjie Jiang⁶, Wenjiao Zhu⁵, Zhian Tang⁶, Zhijun Ge⁷, Tieliang Ma⁵, Yongfei Tan²

Departments of ¹Laboratory, ²Cardiac & Thoracic Surgery, ³Pharmacy, ⁴Respiratory, ⁶Traditional Chinese Medicine, ⁷Critical Care Medicine, ⁵Central Laboratory, The Affiliated Yixing Hospital of Jiangsu University, Yixing, Jiangsu, China. * Equal contributors.

Received October 11, 2014; Accepted January 21, 2015; Epub February 15, 2015; Published February 28, 2015

Abstract: In order to investigate the mechanism of human esophageal Eca109 cells induced by Diosgenin (Dio), the p38 specific inhibitor SB203580 was used to inhibit the expression of p38 and Western blot was employed to detect the effect of SB203580 in Eca109 cells. MTT experiments were executed to detect the proliferation of the cells. Western blot was also applied to find the expression of phosphorylated p38 (p-p38). It is found that SB203580 can inhibit the expression of p38 in human esophageal cell Eca109. After treated with 50 μg/mL of Dio and 10 μg/mL of SB203580, the proliferation of cells showed significantly increase and the apoptosis of cells showed significantly decrease compared with the proliferation in the cells treated with Dio only. Moreover, p-p38 protein level was significantly decreased after treated by the two drugs. It is concluded that Dio may regulate esophageal Eca109 cells through p-p38 pathway.

Keywords: Diosgenin, p38, human esophageal cancer, SB203580

Introduction

Esophageal cancer, with the fourth mortality in tumors, has the high incidence of tumor in the digestive tract tumor. The occurrence and development of esophageal cancer were closely related to various cell signaling pathways [1]. Mitogen-activated protein kinases (MAPKs) are a highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes such as proliferation, apoptosis, differentiation, motility, and stress response. Conventional MAPKs include the extracellular signal-regulated kinase 1 and 2 (Erk1/2), the c-Jun N-terminal kinases 1-3 (JNK1-3): also known as stress activated protein kinases, the p38 isoforms (p38α, β, γ, and δ), and Erk5 [2-4]. As well as other MAPK cascades, the membrane-proximal component is a MAPKKK, typically a MEKK or a mixed lineage kinase (MLK). The MAPKKK phosphorylates and activates MKK3/6, the p38 MAPK kinases [5]. The pyridinylimidazole compounds, exemplified by SB203580, were originally prepared as inflammatory cytokine synthesis inhibitors that subsequently were found to be selective inhibitors of p38 MAP kinase [6].

General surgery and chemotherapy is the main treatment of esophageal cancer. Dio, a kind of plant steroid compounds, could induce the apoptosis of many kinds of tumor cells, and restrain the effect of migration and invasion [7-10]. Based on our previous research, the decrease of the phosphorylated p38 might be one of the mechanisms that Dio affect esophageal Eca109 cells, while the concrete mechanism is still unknown [11]. The objective of our research is the effect of p38 pathway inhibitor and Dio on Eca109 cells, and eventually we can provide new strategies for the treatment of esophageal cancer.

Materials and methods

Materials
Human esophageal Eca109 cell line was purchased from American Type Culture Collection and was cultured in the RPMI-1640 medium.
and CO₂ incubator with 37°C and saturated humidity. The antibodies of p38 and p-p38 were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The antibodies of Caspase3 and GAPDH were purchased from Beyotime Corporation (Nantong, China), and Dio was purchased from Sigma-Aldrich (S8534). SB203580 was purchased from Beyotime Corporation (Nantong, China).

**Methods**

**MTT assay:** Eca109 cells were cultured in 96-well cell culture plate in the density of 1 × 10⁵ each well with six duplications. The cultured RPMI 1640 medium was replaced with mixed medium of Dio (50 μg/mL) or Dio (50 μg/mL) or SB203580 (10 μg/mL). After cultured for 24 and 48 hours respectively, the cells were used for measuring the absorbance at 570 nm (A570) value through Microplate Reader (infiniti F50; Tecan, Männedorf, Switzerland).

**Western blot:** Eca109 cells were seeded into 6-well cell culture plate. The cultured RPMI 1640 medium was replaced with mixed medium of Dio (50 μg/mL) or Dio (50 μg/mL) or SB203580 (10 μg/mL). The negative control group without drugs was set at the same time. The total proteins in each group were extracted and electrophoresed by Western blot, exposed by ECL. The integral optical density of the band was observed with Gel-Pro Analyzer 4.0 software. Protein levels were quantified by relative to tubulin, the software used was Gel-Pro analyzer (Media Cybernetics Inc., Rockville, MD, USA).

**Statistical analysis**

SPSS14.0 software was applied in the process of data statistical processing. The values of the measurement data are expressed as the means ± SD. The equal variance was performed using t test or one way ANOVA. P < 0.05 was considered statistically significant.

**Results**

The p38 special inhibitor SB203580 inhibits p38 expression

After the cells were subjected to SB203580 (10 μg/mL), the level of p38 had a significant decrease compared with the control group, which illustrated that SB203580 has a more obvious inhibitory effect on the protein expression of p38 (Figure 1).

SB203580 inhibits the proliferation of the Eca109 cells

The MTT results revealed that after the cells were subjected to Dio (50 μg/mL), the proliferation of the Eca109 cells were significantly inhibited. With the increase of Dio doses, the inhibitory effect on cell proliferation became more obviously with time (Figure 2).

SB203580 increases the Eca109 cell apoptosis

After the cells were subjected to Dio (50 μg/mL) for 48 h, the expression of Caspase3 protein was evaluated by Western blot analysis. The result revealed that compared with the control group, the expression of Caspase3 had a significantly increase and the effect was more obviously when the dose increased (Figure 3).

SB203580 down-regulated the expression of p38

After the cells were subjected to Dio (50 μg/mL) for 48 h, the expression of one of the MAPK signal pathways (p-p38) was evaluated by Western blot analysis. The result revealed that compared with the control group, the expres-
Role of SB203580 in human esophageal cancer

Discussion

Chinaroot greenbrier genera plants have a very complicated chemical composition. According to the recent and modern plant chemical analysis, its main composition is sapo-nins, and based on Dio as the main saponin glycosides derivatives. According to our previous study, Dio might regulate human esophageal cancer cells Eca109 through p38 signaling [11]. The purpose of our research is to illustrate the reliability of Dio regulated the p-p38 signaling by using the p38 specific inhibitor SB203580.

It is found that after treating the esophageal cancer cells, Dio has various effects on the cell proliferation, apoptosis, migration, and invasion [11]. Our results show that after treating with Eca109 cell, Dio can significantly inhibit Eca109 cell proliferation.

Based on earlier findings, Dio made K562 gene fragment in chronic myeloid original leukemia cell by inhibiting the activation of NF-κB and p38 signaling pathways [13, 14]. Dio induced cell apoptosis by activating p53 signal and regulating Caspsae3 activity in HEL leukemia cell. Dio enhanced the activation of p38 signal [15], but has no effect on the activation of JNK and Erk1/2 in colon cancer cell lines HCT-116 and HT-29 [16]. In non-small cell lung cancer A549 cells, Dio and TRAIL activated MAPK signaling pathways extensively, and the expression of JNK, Erk1/2 and p38 were increased dramatically [17]. Another study found that p38 regulated the apoptosis of human hepatoma HepG2 cells via p53, BAX and Caspase9 et. al [18]. In our study, compared with negative control group, the protein level of p-p38 had significantly decreased after treatment with Dio after the three proteins in the MAPK pathways were examined. The p38 special inhibitor SB203580 inhibited the proliferation of the Eca109 cells and increased the Eca109 cell apoptosis, while down-regulated the expression of p38. These results suggest that Dio regulated cell migration and invasion by inhibiting p38 protein.

In conclusion, we consider that Dio might regulate the esophageal cancer Eca109 cells through p38 signaling pathways, while the upstream and downstream mechanism are still unclear and need further study.
Role of SB203580 in human esophageal cancer

Acknowledgements

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This work was supported by Fund of Science and Technology of Yixing (2013-15) (2013-21) and Fund of Clinical Science and Technology of Wuxi (ML201304).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yongfei Tan, Department of Cardiac & Thoracic Surgery; Dr. Tieliang Ma, Central Laboratory, The Affiliated Yixing Hospital of Jiangsu University, #75 Tongzhenguan Road, Yixing 214200, Jiangsu, China. Tel: +86-510-87921196; Fax: +86-510-87921110; E-mail: yongfeitan@sina.com (YFT); matieliang@foxmail.com (TLM)

References


