ELSEVIER MASSON

Available online at www.sciencedirect.com

Biomedicine & Pharmacotherapy 62 (2008) 583–589

BIOMEDICINE & PHARMACOTHERAPY

www.elsevier.com/locate/biopha

Dossier : Molecular and cellular effects of nutrients : global perspectives and modern aspects

Apoptotic activity of genistein on human lung adenocarcinoma SPC-A-1 cells and preliminary exploration of its mechanisms using microarray

Huafei Zou, Shuxuan Zhan, Kaiming Cao*

State Key Laboratory of Genetic Engineering, Department of Biochemistry, School of Life Sciences, Fudan University, 220 Handan Road, Shanghai 200433, PR China

Received 8 November 2007; accepted 12 December 2007
Available online 29 January 2008

Abstract

Soy isoflavone genistein is active against certain solid malignancies, but its direct effect on lung adenocarcinoma and its mechanisms of action remain to be elucidated. In the present study, using the human lung adenocarcinoma cell line SPC-A-1, we found that genistein decreased SPC-A-1 cell viability in both a dose and time dependent manner. Flow cytometry analysis revealed that genistein significantly induced arrest of SPC-A-1 cells at the G2/M phase of the cell cycle. Furthermore, through DNA fragmentation and TUNEL assays, we demonstrated that the addition of genistein led to SPC-A-1 apoptosis in both a dose and time dependent manner. Finally, the apoptosis pathway-related gene expression profile affected by genistein was investigated using the oligonucleotide microarray method. The result showed that the expression profile of 20 genes (ratio of genistein group/control group >2 or <0.5) related to the apoptotic pathways changed. These genes, mainly consisting of the Bcl-2 family and TNF ligand and receptor family, are involved in regulation of the apoptosis process.
© 2007 Published by Elsevier Masson SAS.

Keywords: Genistein; Lung adenocarcinoma; Apoptosis; Oligonucleotide microarray

1. Introduction

Lung cancer is one of the leading causes of cancer death. Despite the combination of available therapeutic treatments, including surgical, radio, and chemo therapies, the 5-year survival rate of lung cancer is only 15%, compared to a 62–97% average in other common human cancers [1–3]. Therefore, there is an urgent need to develop novel preventive and therapeutic strategies with a better understanding of their molecular mechanisms against lung cancer.

Nutritional chemoprevention is an attractive therapeutic approach that has already been shown to be effective in the treatment of many cancers. Among those substances, the soy isoflavone genistein is of great interest because it decreases the associated risk of tumor initiation and inhibits the growth of breast, prostate and colon cancer [4–6]. Several studies suggest that genistein is also active against lung cancer [7–9]. Lian et al. found that genistein induced apoptosis in non-small-cell lung cancer cell lines H460 and H322 [10]. However, few investigations have analyzed its direct effect on lung adenocarcinoma, which constitutes 40% of lung cancers [3]. In addition, the molecular mechanism under which genistein acts against lung adenocarcinoma remains to be elucidated.

Growing evidence has shown that the soy isoflavone genistein has the ability to inhibit cancer cell proliferation and induce cell apoptosis through several molecular mechanisms, such as the regulation of androgen receptor (AR), NF-kappa B, Akt and MAPK pathways [11–15]. Genistein is known as an antioxidant, which regulates stress-modified signal transduction [16]. It also inhibits DNA topoisomerases and other critical enzymes involved in signal transduction [17,18]. Furthermore, genistein arrests cells at the G2/M phase

* Corresponding author. Tel./fax: +86 21 65643672.
E-mail address: kmcao@fudan.edu.cn (K. Cao).

0753-3322/$ - see front matter © 2007 Published by Elsevier Masson SAS.
doi:10.1016/j.biopha.2007.12.010

of the cell cycle, thus inhibiting cell proliferation [19]. Genistein has also been shown to stimulate apoptosis in several cancer cell lines [10,20]. Apoptosis after exposure to genistein can be a consequence of the above mentioned oxidative stress or the inhibition of enzymes involved in cell proliferation [21]. However, the precise mechanism is still not clear.

In this study, the effects of genistein on cell viability, cell cycle and apoptosis of lung adenocarcinoma cell line SPC-A-1 were evaluated both in a time and dose dependent manner. To further understand the action of genistein, the apoptosis pathway-related gene expression profile was investigated using the oligonucleotide microarray methodology.

2. Materials and methods

2.1. Cell culture

Human lung adenocarcinoma SPC-A-1 cells were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China) and were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 U penicillin/streptomycin at 37 °C with 5% CO_2 in air atmosphere. The cells were treated with different concentrations of genistein for different times as indicated in Section 3. A 200 mM genistein (Sigma) stock solution was prepared in dimethylsulfoxide (DMSO) and then diluted with RPMI 1640 to get the final concentrations. We used DMSO in RPMI 1640 as a control.

2.2. MTT assay

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed to quantify cell growth and proliferation. Cells were initially seeded in 96-well microplates at the density of 4×10^3 cells/well. After genistein treatment, 10 µl MTT (5 mg/ml) was added to each well and the plates were incubated at 37 °C for another 4 h. The purple formazan crystals were dissolved in 150 µl DMSO, and absorbance was determined at 570 nm using an autoreader (BioRad).

2.3. Cell cycle analysis

Non-synchronized cells were exposed to genistein and then harvested by trypsinization, washed in PBS and fixed in 75% ethanol at 4 °C for 2 h. Subsequently these fixed cells were incubated with 200 µg/ml RNase (Sigma) and 50 µg/ml propidium iodide (Sigma) at 37 °C for 30 min in the dark. The cell cycle was analyzed by flow cytometry (FACScan; Becton Dickinson).

2.4. Low-molecular-weight DNA isolation

Cells were harvested and incubated at 53 °C for 8 h in a buffer containing 0.2 mg/ml proteinase K, 100 mM Tris–HCl, pH 8.0, 0.1 M EDTA and 0.5% SDS. DNA was purified by extraction with equal volumes of phenol. The aqueous phase was then ethanol precipitated for 2 h at −70 °C, pelleted, and resuspended in TE buffer. The resulting DNA was digested with DNase-free RNase. A total of 30 µg DNA was loaded on a 2% agarose gel and separated by electrophoresis. DNA was stained with ethidium bromide and visualized with an ultraviolet transilluminator.

2.5. TUNEL assay and quantitation

Cells undergoing apoptosis were determined by using TUNEL staining. Briefly, the cells were fixed with freshly diluted 4% paraformaldehyde in PBS for 20 min, followed by washing with PBS twice and quenching in 3% hydrogen peroxide. TUNEL assay was then performed with an ApopTag Kit (Intergen) according to the manufacturer's instruction. The stained cells were mounted and observed under light microscopy. To assess the incidence of apoptosis, the cells were quantified by counting about 300 cells in five to seven separate random fields of view per slide.

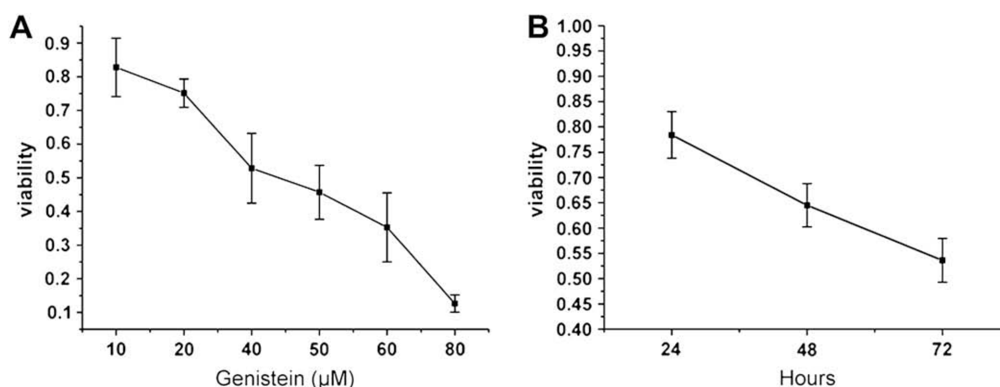

Fig. 1. Genistein inhibited SPC-A-1 cell growth in a dose and time dependent manner. (A) Cells were treated with various concentrations of genistein and the viability was assessed by MTT assay after 72 h. (B) Genistein (40 µM) was added to the cells for different days. Data were reported as the percentage of the viability of the genistein-treated group compared to the vehicle-treated group. The values were expressed as mean ± SD and three separate experiments were repeated.

Download English Version:

https://daneshyari.com/en/article/2525493

Download Persian Version:

https://daneshyari.com/article/2525493

Daneshyari.com