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Lycium barbarum polysaccharide stimulates proliferation of MCF-7 cells by the ERK pathway

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ABSTRACT

Aims: The aim of this study was to investigate the anti-proliferative effect of *Lycium barbarum* polysaccharide (LBP) on MCF-7cells.
Main methods: MCF-7 cells were treated with 0, 10, 30, 100, and 300 μg/ml LBP for 24 h. The cell cycle distribution was analyzed by flow cytometry. MEK inhibitor, U1206 also was added in MCF-7 cells to deal with LBP (300 μg/ml) for different times (0, 2, 4, 6, 8, 16, 24 h). Western blotting was used to indicate changes in the levels of ERK (extracellular signal-regulated protein kinase) and phosphorylation-ERK (p-ERK) to compare the relationship between ERK activity and LBP. The expressions of p53, p-p53 and p21 were observed by western blotting to analyze the relationship between p53 and ERK which was under the treatment of LBP.
Key findings: The MCF-7 cell cycle was arrested in S phase with the treatment of LBP. The LBP can also activate ERK, which may be associated with p53 pathway. There was a dose-dependent increase in the relation between the degree of ERK activation and LBP.
Significance: LBP induces the anti-proliferation of MCF-7 cells by activating ERK.

Introduction

Fruit from *Lycium barbarum L.* in the family Solanaceae is well known in traditional Chinese herbal medicine (Potterat, 2010), and nowadays it is used widely as a popular functional food (Li, 2007). It has stimulated great interest in current research because of its immunomodulation and antitumor activity. *L. barbarum* polysaccharide (LBP) has been identified as the major active ingredient, which was isolated from aqueous extracts of *L. barbarum*. Its activities include antiaging (Chang and So, 2008) effects, antioxidant (Potterat, 2010) properties, increased metabolism (Amagase and Nance, 2008), antitumor activity (Mao et al., 2011; Luo et al., 2009), promotion of endurance and so on. In our previous work, we found that LBP could enhance the proliferation of splenic lymphocyte (Du et al., 2004) in mice.

Accumulating evidence both in vitro and in vivo suggests that the anticancer effects of LBP may be attributed to its immunomodulatory property and activate T cell, B cell, and NK cell levels to suppress the growth of tumor cells. Relevant literature reports that LBP could not only inhibit the proliferation of HL-60 cell (Gan et al., 2001) by decreasing membrane fluidity but also improve the expression level of IL-2 and TNF-α in human peripheral blood mononuclear cells (Gan et al., 2003).

Anti-tumor effects could be attributed to altered biochemical mechanisms, including inhibitions of proliferation, induction of cell cycle arrest at various cell cycle checkpoints, enhanced apoptosis, and regulation of signal transduction pathways (Swanton, 2004). Apoptosis is regarded as the preferred way and is one of the key pathways to manage cancer (Hengartner, 2000). LBP could inhibit the proliferation and induction of apoptosis on human hepatoma QGY7703 cells (Zhang et al., 2005) by the increase of intracellular calcium in an apoptotic system. LBP could promote cell cycle arrest at the G0/G1 or S phase in colon cancer cells (Mao et al., 2011), MGC-803 and SGC-7901cells (Miao et al., 2010) which are derived from the same type of human gastric cancer cell, respectively.

Breast cancer is one of the most common cancers for women worldwide (Jemal et al., 2008). A recent study suggests that it suppresses IGF-1-induced angiogenesis via PI3K/HIF-1α/VEGF signaling pathways (Zhang et al., 2012) in MCF-7 cells. ERK (extracellular signal-regulated protein kinase) is an important regulator of cell proliferation (Chambard et al., 2007). Activation or inhibition of ERK signal transduction cascades has been shown to induce cell growth (McCubrey et al., 2007), differentiation or apoptosis in a number of tissues, including breast cancer (Helle, 2004).

Therefore, we examined the effect of LBP on cell cycle distribution and apoptosis in the breast cancer cell line, MCF-7. Furthermore, to explore the anti-breast-cancer mechanism of LBP, we determined the levels of ERK and p-ERK, which are the possible signal transduction pathways of apoptosis to anticancer agents.

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Materials and methods

Materials

DMEM (Dulbecco's modified Eagle's medium) was obtained from GIBCO BRL. FBS (fetal bovine serum), penicillin G and streptomycin were products from Sigma (St. Louis, MO, USA). Anti-ERK and anti-phospho-ERK (1:500) were obtained from New England BioLabs (NEB). Anti-p53 (FL-353 at 1 μg/ml), anti-$p21^{CIPI}$ (1 μg/ml) and anti-Actin were purchased from Santa Cruz Biotechnology. Anti-phospho-p53 (S15, 1:1000) was obtained from Cell Signaling.

Preparation of LBP

LBP was supplied by Shanghai Organic Chemistry Institute. LBP was prepared as a stock solution of 5000 mg/l in basal medium DMEM and kept at −20 °C. For all experiments, final concentrations were prepared by diluting the stock solution with DMEM. Control cultures received the same volume of DMEM.

Cell culture and treatments

MCF-7 cells were purchased from the American Type Culture Collection (ATCC). MCF-7 cells were maintained in DMEM media supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 10 μg/ml streptomycin and cultured at 37 °C in a humidified incubator (SANYO) with 5% CO_2.

Analysis of cell-cycle phase distribution by flow cytometry

MCF-7 cells (2×10^5 per well) were seeded into 6-well plates and were treated with LBP (10, 30, 100, and 300 μg/ml) for 24 h, while the control group received only DMEM. The cells were collected, washed by PBS for three times and were fixed with ice-cold 70% ethanol. Then the mixture was analyzed by flow cytometer (Beckman). The percentage of cells in the three lines of growth cycle (G0/G1, G2/M and S phase) was estimated using the Cell Quest software system.

Western blotting

Western blotting was performed as described previously (Guo et al., 2008). For the western blot analysis, MCF-7 cells were treated with LBP as above then 2×10^5 cells were lysed in RIPA buffer (50 mM Tris–HCl, 20 mM β-ME, pH 7.5, 5 mM EDTA, 150 mM NaCl, 0.5% Triton X-100). The lysates were boiled and separated by 12% SDS-PAGE gel and electroblotted onto nitrocellulose membranes. In this study, the primary antibodies used for western blotting were anti-ERK and anti-phospho-ERK. The signals were visualized using the ECL method.

ERK inhibition test

MCF-7 cells were cultured in 300 mg/ml LBP-DMEM medium, with 10 μM U1206. The control group was without U1206. At intervals of 0, 2 h, 4 h, 8 h, 16 h, and 24 h after the addition of LBP, the expressions of ERK, p-ERK, p-p53, p53 and p21 were tested as detailed above.

Statistics

Data were listed as mean ± SD. One-way ANOVA was used to make a statistical comparison between groups. P value of less than 0.05 was considered statistically significant.

Results

Effect of LBP on cell cycle progression of MCF-7

Since cell growth and inhibition are both tightly regulated by the cell cycle control (Sanchez and Dynlacht, 2005), the changes in cell-cycle distribution were analyzed by flow cytometry (Fig. 1). MCF-7cells were treated with 10, 30, 100 and 300 μg/ml LBP for 12 h. The cell cycle analysis showed that there was a progressive dose-dependent decrease in the distribution of cells in G0/G1 phase (49.06% to 22.68%). A significant increase in the index of cells in S phase (45.29% to 71.10%) was also observed in various LBP concentrations, whereas 28.99% in media control cells. The results showed LBP arrested the MCF-7 cells in the S phase.

Effects of LBP on activation of ERK

The phosphorylation of ERK and ERK protein of MCF-7 cells which were stimulated with LBP (10, 30, 100 and 300 μg/ml) was analyzed by Western blotting. The results indicated that p-ERK was activated after treatment with LBP, but there was no effect on ERK level (Fig. 2A). LBP also activated the level of p-ERK in MCF-7cells in a dose-dependent manner (Fig. 2B). Further studies will be required to validate the involvement of ERK pathways in LBP-mediated reductions in MCF-7cells.

Effects of MEK inhibitor on LBP

MEK inhibitor was used to investigate the involvement of ERK in the mechanism of LBP. The U0126 was added to the cultured MCF-7 cells and 300 μg/ml LBP for 2, 4, 8, 16, and 24 h time intervals were used for further studies. At the same time, the control group was without U0126. In the group with U0126, LBP-treatment inhibited the level of p-ERK expression in MCF-7 cells. However, there was no effect on ERK level (Fig. 3). The results suggest that LBP suppressed the cell cycle of MCF-7 cells depending on the activation of ERK.

The levels of p53 and p21

Since the inhibitory mechanism of LBP on cell proliferation may be caused by the expression of negative regulators of the cell cycle, we evaluated the protein expression of p53, p-p53 and p21. As shown in Fig. 3, the levels of p53 and p-p53 were obviously increased by LBP in MCF-7 cells. The signaling was initiated by p21 that results in the assembly of the kinase cascade, particularly the ERK pathway (Tsujita et al., 2008). The expression of p21 was also markedly up-regulated by treatment with LBP in a time dependent manner (Fig. 3). With U0126, the levels of p53, p-p53 and p21 were all decreased by LBP. The ERK pathway has been implicated in the induction of the expression of the p21. These observations show that p53 transactivation activity was similar to ERK activation. P53 may act as one of the upstream regulators of ERK activation for the induction of apoptosis in LBP treated MCF-7 cells.

Discussion

At present the main clinical tumor therapy methods are surgery, radiotherapy and chemotherapy, but these have some side effects. In recent years, attention to natural therapies has aroused a new wave of research interest in traditional practices. Derivatives from plant extracts have become targets for the search of new anticancer drugs (Butler, 2008). Paclitaxel and camptothecin are the best examples. LBP, which is a kind of polysaccharide–protein complex isolated from the traditional Chinese herb *L. barbarum*, has anticancer and immunologic enhancement activities. It has been reported that the *L. barbarum* juice, whose main active compound is LBP, increased