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Anti-fibrotic effects of Ginsan

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1. Introduction

Pulmonary fibrosis is the consequence of a variety of diseases with no satisfying treatment option. Therapy-induced fibrosis also limits the efficacy of chemotherapy and radiotherapy in numerous cancers (1). It has been proposed that fibrogenesis is not a unique pathologic process but rather, is due to an excess of the same biologic events involved in normal tissue repair (2). Persistent and exaggerated wound healing ultimately leads to an excess of fibroblast replication and matrix deposition (1, 3). Several studies revealed that TGF-β1, collagen 1, fibronectin, various chemokine and some anti-oxidant are overexpressed in radiation-induced pulmonary fibrosis (4).

A number of studies were performed that polysaccharide extracted from Panax ginseng C.A. Meyer, ginsan, has been demonstrated to be a potent promising biological response modifier (BRM), including proliferation of lymphocytes, generation of lymphokine activated killer cells, and production of several cytokines (5-7). On the basis of several results of the ability of ginsan on modulation of redox system and cytokine balance, we examined whether ginsan directly regulates fibroblast proliferation, differentiation factors, and also investigated the mechanism of the anti-fibrotic effects of ginsan.

2. Materials and Methods

2.1 Cell culture

NIH-3T3 cells and human lung fibroblast cell line (WI38) were cultured in DMEM, and human bronchial epithelial cell line BEAS 2B were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 0.1% penicillin/ streptomycin at 37℃ under 5% CO_2 humidified atmosphere.

2.2 Cell proliferation assay

NIH-3T3, WI38 or BEAS2B cells seeded onto a 96-well plate at a density of 3000 cells per well and allowed to attach overnight. The medium was then changed to a serum-free medium. After 24h starvation, the cell were re-stimulated with 10% FBS and treated with 5ng/ml TGF-β1. The cells were treated with various concentrations of ginsan 3 h after TGF-β1 stimulation, then incubated for 2 days and counted with the Cell Counting Kit-8 according to the manufacturer's instructions.

2.3 RNA isolation and quantitative RT-PCR

Total RNA was extracted by acid guanidium thiocyanate-phenol-chloroform extraction method with Trizol. One microgram of intact total RNA was reversibly transcribed into first strand cDNA, which was then amplified using polymerase chain reaction (PCR). The final volume of 20 μl of reverse transcriptase (RT) reaction mixture contained; 50 mM Tris-HCl (pH 8.3), 3 mM MgCl2, 75 mM KCl, 2.5 μg /μl pd$(N)_6$ primer, 0.5 mM each of dNTP and 10 U of AMV-RT (Amersham, USA). The reaction mixture for PCR contained 10 $\mu\ell$ of cDNA template from RT reaction, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.5 mM each of dNTP mixture, 1.0 μM each of primer and 0.5U Taq DNA polymerase (Takara, Japan). PCR was performed with GeneAmpR PCR system 9700 (Applied Biosystems, USA) at 94°C for 1 min, at 55 ~ 60°C for 1 min, and at 72°C for 1 min per cycle. The numbers of amplification cycles were determined according to individual primer set in order to maintain exponential rate of product amplification (28 - 35cycles). The amplified products were visualized by electrophoresis on a 1% agarose gel in the presence of 0.5 μg /μl ethidium bromide, and the density of bands was quantitated by Image analyzer (Fluor-S™ multiImager, Bio-Rad, USA).

3. Results

3.1 Ginsan inhibited cell proliferation on TGF-β1 treated cells

To analyze whether ginsan exerted anti-fibrotic effects, we first investigated the effect of ginsan on cell proliferation in fibroblasts and epithelial cells. No significant difference was observed between the ginsan alone and control groups in all cell lines, but ginsan markedly inhibited the proliferation of fibroblasts in the

presence of TGF- β1. In contrast, the treatment of ginsan did not change the cell survival on epithelial cells with or without TGF- β1.

3.2 Post-treatment with ginsan inhibits TGF-β1 and TGF receptor 2 mRNA expressions in NIH-3T3 cells

To investigate the inhibitory effect of ginsan on TGF-β1 and its related molecules in fibroblasts, NIH-3T3 cells were treated with TGF-β1 in the presence or absence of ginsan and determined the mRNA expressions of TGF- β1, TGFR-1, TGFR-2, Col I and α-SMA by the RT-PCR. Ginsan significantly decreased TGF- β1 and TGF-β receptor-2 mRNA expression in NIH-3T3 cells.

4. Conclusion

In the present study, we examined whether ginsan affected the function of fibroblasts or epithelial cells. Our results revealed that ginsan suppressed TGF-β1 induced collagen 1, TGF-β receptor-1 and -2 mRNA and inhibit fibroblast proliferation in the presence of TGF-β1. In contrast, the epithelial cells treated with ginsan did not exhibit any changes, suggesting that ginsan could be act differently in the processes of the development of fibrosis in vivo.

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