Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 268–276
Phytomedicine
www.elsevier.de/phymed

Differential protein expression in mouse splenic mononuclear cells treated with polysaccharides from spores of *Ganoderma lucidum*

Chao Ma, Shu-Hong Guan, Min Yang, Xuan Liu*, De-An Guo*

Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

Abstract

Polysaccharides were one of the main components of *Ganoderma lucidum*, a medicinal mushroom well known for its immuno-modulation effects. In the present study, we demonstrated that polysaccharides extracted from *Ganoderma lucidum* spores (GL-SP) could stimulate splenic mononuclear cells (MNCs) proliferation and cytokine production. To identify the possible cellular targets of GL-SP in MNCs, two-dimensional gel electrophoresis (2-DE)-based comparative proteomics was performed and proteins altered in expressional level after GL-SP treatment were identified by MALDI–TOF MS/MS. Ten proteins with >2-fold increase or decrease expression in GL-SP-treated MNCs compared with control were found and further identified by MALDI–TOF MS/MS analysis and database searching. In the GL-SP-treated MNCs, there were increases in the expression of myosin regulatory light chain 2-A, Rho GDP dissociation inhibitor beta, T-cell-specific GTPase, phosphatidylinositol transfer protein α, and decreases in the expression of apoptosis-associated speck-like protein containing a CARD, 14-3-3 tau, beta-actin, tubulin alpha 2, copine I, and gamma-actin. The results of the present study help to provide insight into the immuno-modulating activities of GL-SP.

Keywords: *Ganoderma lucidum*; Polysaccharides; Proteomics; Mononuclear cell; Immuno-modulation

Introduction

The *Ganoderma lucidum* (Leyss ex fr) Karst (Lingzhi) is a medicinal mushroom that has been used for more than 2000 years as a remedy for promotion of health and longevity in China and other Asian countries. It is now also popular as a dietary supplement in the form of tea powder or extract in Western countries. Considering that the water-soluble extract is the most popular form of traditional Chinese medicine, the activity and mechanism of polysaccharides, which are the main components in water-soluble extract of *G. lucidum*, is a major area of research about *G. lucidum*. In the recent 30 years, modern pharmacological research about *G. lucidum* polysaccharides clearly demonstrated its immuno-modulating and anti-tumor activities (Lin, 2005; Paterson, 2006). The immuno-modulating effects of *G. lucidum* polysaccharides were extensive, including promoting the function of mononuclear phygocyte system, humoral immunity, and cellular immunity. There have been many reports that demonstrated that *G. lucidum* polysaccharides can induce cytokine production and differentiation of lymphocytes (Wang et al., 1997; Hsu et al., 2004), maturation of cultured murine bone marrow-derived dendritic cells and the immune response initiated by dendritic cells (Cao and Lin, 2002),

*Corresponding authors. Tel./fax: +86 21 50271516.
E-mail addresses: lillianliucn@yahoo.com.cn (X. Liu), daguo@mail.shcnc.ac.cn (D.-A. Guo).

0944-7113/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.11.015

proliferation and immuno-globulin production of murine splenic B cells (Lin et al., 2006) and natural killer cells activation (Chien et al., 2004) and finally result in the activation of immune system. However, molecular mechanisms for the immuno-biological function of *G. lucidum* polysaccharides are far from clear.

In this paper, we found that GL-SP can stimulate mouse splenic mononuclear cells (MNCs) proliferation and increase the production of IL-2 and TNF-α in MNCs. For a comprehensive analysis of the immunomodulating mechanisms of GL-SP, a proteomic approach (two-dimensional electrophoresis (2-DE) and mass spectrometry (MS)) was used for seeking differential protein expression in MNCs treated with GL-SP. The term ''proteome'' refers to all proteins and their contents in cells under a given condition. Using of proteomic approach offers us the opportunity to characterize global alterations in protein expression of MNCs treated with GL-SP.

Materials and methods

Extraction and chemical characterization of GL-SP from spores of *G. lucidum*

Spores of *G. lucidum* were collected in August 2005 from Wu Yi Mountain (Fujian Province) GAP cultivation base of Green Valley Pharmaceutical Co. Ltd., People's Republic of China. Samples were deposited in Shanghai Research Center for Modernization of Traditional Chinese Medicine (200508004). GL-SP was extracted from the spores of *G. lucidum* using a method similar to the standard methodology of (Huie and Di, 2004). Briefly, the spores of *G. lucidum* (100 g) were defatted with 95% alcohol and then refluxed with 20 volumes of water. The aqeous extract was fractionated into a polysaccharide fraction (alcohol insoluble) and nonpolysaccharide fraction (alcohol soluble), and further treated with trichloroacetic acid to remove proteins, and dialyzed against tap water for 2 days and distilled water for 1 day (molecular weight cut-off 3000–5000). The retentate was washed sequentially with EtOH and acetone and concentrated under vacuum and freeze dried to obtain the polysaccharides as a yellow-white powder (1 g). GL-SP was dissolved in RPMI-1640 medium, filtered through a 0.22 μm filter and stored at −20 °C before use in biological study.

The chemical characteristics of GL-SP were analyzed using HPLC, IR and elemental analysis methods as reported before (Wang et al., 2007). Briefly, the HPLC analysis was performed on an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, a diode-array detector (DAD), an autosampler, and a column compartment. The sample was separated on a Zorbax SB-C_{18} column (5 μm, 4.6 mm × 150 mm, Agilent). The mobile phase consisted of acetonitrile (CH_3CN) and water containing KH_2PO_4 (pH 5.0), eluted with 16.5% CH_3CN over the first 25 min, then to 24% in 30 min. The flow rate was 1.0 ml/min, and column temperature was set at 45 °C. The DAD detector was monitored at 245 nm, and the on-line UV spectra were recorded in the range of 190–400 nm. The optical rotation was measured on a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet 750 spectrometer. The elemental analysis was performed on a UarioEL elementor. The representative HPLC analysis figures are shown in Fig. 1. The GL-SP was hydrolyzed by trifluoroacetic acid (TFA) and then derivatized by 1-pheny-3-methyl-5-pyrazolone (PMP) before HPLC analysis. As shown in Fig. 1, seven monosaccharides (D-mannose, D-ribose, D-glucose, D-galactose, L-arabinose, D-xylose, and L-fucose) in GL-SP were identified by comparing with their reference standards. The essential components of GL-SP were glucose (about 77%), galactose (about 12%) and mannose (about 6%), while other monosaccharides were not more than 5%. The specific rotation $[\alpha]_D^{23}$ was $+21.7^\circ$ (ca. 0.1385, H_2O). The character absorption at ca. 890 cm^{-1} in the IR spectra of GL-SP indicated that the glycosyl residues were linked mainly by β glycosidic linkage. The N-content, C-content, and H-content of the polysaccharides were 1.18%, 37.40%, and 6.61%, respectively. The ratio of polysaccharides to peptide was about 93.5:6.5%. Similar to previous reports (Bao et al., 2002, Jiang et al., 2005; Wang et al., 2007), our results indicated that polysaccharides extracted from *G. lucidum* were heteropolysaccharides with D-glucose as the main monosaccharide component, and the ratio of polysaccharides to peptide was about 3.5–5%.

Animals and reagents

Inbred KM mice (male, 6 weeks old) were fed and kept in the Shanghai Experimental Animal Laboratory, Chinese Academy of Sciences. All procedures performed in animals were performed under the control of the Institutional Animal Care and Use Committee. All reagents used in 2-DE were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Other chemicals, except where specially noted, were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

Preparation of mouse splenic mononuclear cells

Mice were sacrificed by cervical dislocation and the spleens were removed aseptically. Single-cell suspension was prepared by filtering the spleens through a stainless steel 200-mesh using a syringe plunger. Splenic MNCs