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Research article

A proteomic approach reveals the differential protein expression in *Drosophila melanogaster* treated with red ginseng extract (*Panax ginseng*)

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ABSTRACT

Background: Red ginseng is a popularly used traditional medicine with antiaging effects in Asian countries. The present study aimed to explore the changes in protein expression underlying the mechanisms of life span extension and antiaging caused by red ginseng extract (RGE) in *Drosophila melanogaster*.

Methods: A proteomic approach of two-dimensional polyacrylamide gel electrophoresis (2-DE) was used to identify the differential abundance of possible target proteins of RGE in *D. melanogaster*. The reliability of the 2-DE results was confirmed via Western blotting to measure the expression levels of selected proteins. Proteins altered at the expression level after RGE treatment (1 mg/mL) were identified by matrix-assisted laser desorption/ionization-time of flight tandem mass spectrometry and by searching against the National Center for Biotechnology nonredundant and Uniprot protein databases. The differentially expressed proteins were analyzed using bioinformatics methods.

Results: The average survival life span of *D. melanogaster* was significantly extended by 12.60% with RGE treatment (1 mg/mL) compared to untreated flies. This followed increased superoxide dismutase level and decreased methane dicarboxylic aldehyde content. Based on the searching strategy, 23 differentially expressed proteins were identified (16 up-regulated and 7 down-regulated) in the RGE-treated *D. melanogaster*. Transduction pathways were identified using the Kyoto Encyclopedia of Genes and Genomes database, and included the hippo and oxidative phosphorylation pathways that play important roles in life span extension and antiaging process of *D. melanogaster*.

Conclusion: Treatment with RGE in *D. melanogaster* demonstrated that mechanisms of life span extension and antiaging are regulated by multiple factors and complicated signal pathways.

1. Introduction

Aging, a spontaneous and complex process with the passage of time, is one of the most important risk factors for increasing susceptibility to multiple diseases [1]. It involves much morphological and functional deterioration in biological systems, which are associated with various molecular, cellular, and organic changes [2]. Aging features a progressive accumulation of oxidative agents associated with decreased efficiency of antioxidant defense mechanisms. Related to this is a progressive decrease in immune activity or immune senescence. As animals age, their mitochondrial function and thus cellular metabolism systematically decline, leading to a loss of cellular homeostasis and the occurrence of multiple disorders [3,4]. Thus, a number of efforts have been made to elucidate the mechanisms of the processes of aging and to discover new compounds that retain antiaging activities.

Ginseng (*Panax ginseng* Meyer, Araliaceous) is an important medicinal herb that has long been used to treat various diseases in Asian countries (i.e., Korea, China, and Japan) [5]. Among several kinds of *P. ginseng* products, red ginseng, produced by steaming and drying fresh ginseng, is a main active ginseng. During this process, ginsenosides undergo chemical changes that have the potential to

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create special physiologic activities *in vivo* [6]. This is known to have various physiological activities, including—but not limited to—antioxidant, anticancer, tonic, and antiaging effects. Red ginseng improved learning and memory of the mice [7], and Korean Red Ginseng tonic extended the life span of *Drosophila melanogaster* [8]. However, there is very limited information on the proteomic mechanisms underlying the antiaging effect and life span extension of red ginseng on *D. melanogaster* or other animal models, because existing studies have primarily focused on pharmacology and/or pharmacodynamics association studies [9,10].

In this study, *D. melanogaster*, an internationally recognized model for studying life span that is widely used in antiaging medical research, was used to elucidate this issue *in vivo*. A proteomic approach of two-dimensional polyacrylamide gel electrophoresis (2-DE) was used to investigate changes in age-related specific indicators and relative abundance of proteins in expression caused by feeding with red ginseng extract (RGE). Our findings could be helpful in revealing the mechanisms and addressing biological questions underlying the effects of red ginseng on life span and antiaging.

2. Materials and methods

2.1. Plant materials and reagents

Fresh ginseng (6 yr) was obtained from Jilin City, Jilin Province, China. Red ginseng and its extract (RGE) were processed and provided by the Medicinal Plant Analysis laboratory of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences (Jilin, China), according to the national standard of China (Technical specifications for red ginseng processing, NO. NY/T 2784-2015). The panaxoside content (Fig. 1, Table S1) in the RGE was determined as previously described [11], using high-performance liquid chromatography, as follows (all in mg/g): Rb1 30.13, Rb2 14.67, Rb3 8.23, Rc 20.45, Rd 15.57, Re 16.44, Rf 1.83, Rg1 4.47, Rg2(s) 1.96, Rg2(r) 0.42, Rg3(s) 2.06, Rg3(r) 1.18, Rg5 3.31, Rk1 1.07, and Ro 4.09.

Urea, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS), Pharmalyte, dithiothreitol (DTT), and iodoacetamide were obtained from AMRESCO (Solon, OH, USA). ReadyPrep 2-D cleanup kit and reagents used for 2 DE and Western blotting were purchased from Bio-Rad (Richmond, CA, USA). The primary antibodies for Western blotting included rabbit anti-14-3-3 zeta (1433Z) polyclonal antibody, rabbit antivacuolar-type H(+)-ATPase (VATA) polyclonal antibody, rabbit antiheat-shock protein 70 (HSP70) polyclonal antibody, rabbit antiglycerol-3-phosphate dehydrogenase (GPDA) polyclonal antibody, mouse antiubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) monoclonal antibody mouse antifructose-bisphosphate aldolase (ALF) monoclonal antibody, and mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (dilution 1:1000; Proteintech, Chicago, IL, USA). The secondary antibody used in this study was horseradish peroxidase-conjugated goat antirabbit/mouse IgG (dilution 1:2,000; Proteintech).

2.2. Life span analysis of D. melanogaster

Wild type *D. melanogaster* (Oregon K) was a gift from Jilin Agricultural University and was used throughout the experiment. Single populations (200 flies each) of males were housed in a biochemical incubator with 12-h dark/light cycle at 25°C, 60% humidity, and with free access to basal food (water, 1.2% agar, 2.2% sucrose, 1.8% yeast extra powder, 8% corn extract, and 6.25 μL/L propionic acid). For RGE treatment, basal food was supplemented with RGE at a final w/v concentration of 1 mg/mL optimized by our previous study (unpublished). The food was changed every 2–3 d.

To examine the effects of RGE on the life span of flies, survival was observed and documented at 8:00 P.M. when transferring to fresh basal food or basal food supplemented with RGE every 2–3 d. Survivorships were scored regularly and eliminated the unnatural death flies, and death flies were removed out of the cage when

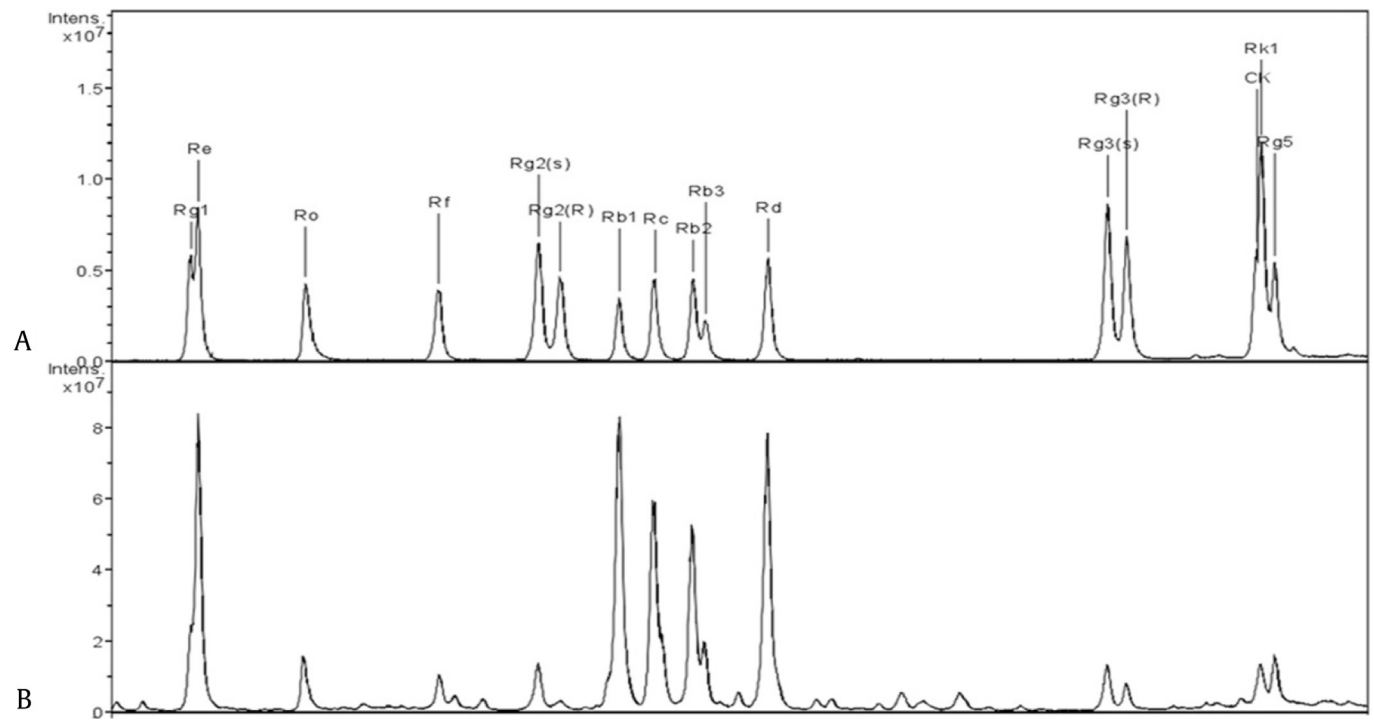

Fig. 1. Panaxoside content of red ginseng extract (RGE) determined by high-performance liquid chromatography (HPLC). (A) HPLC chromatogram of ginseng saponin standard. (B) HPLC chromatogram of RGE.

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