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Proteomic analysis of β-asarone induced cytotoxicity in human glioblastoma U251 cells

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ABSTRACT

Though rhizoma acori graminei (RAG) is frequently prescribed in formulations for brain tumor in traditional Chinese medicine, the potential mechanisms are still unclear. The aim of this study is to determine the effect of β-asarone, a major component in the volatile oil of RAG, against brain tumor and elucidate the underlying molecular mechanisms. The results showed that β-asarone significantly inhibited the cell viability of human glioblastoma U251 cells. Moreover, YO-PRO-1/PI staining revealed that cells treated with β-asarone underwent apoptotic and necrotic death. Then, the two-dimensional gel electrophoresis (2-DE)-based proteomics was applied to investigate the different protein profiles of U251 cells treated with vehicle or β-asarone. Sixteen proteins affected by β-asarone were successfully identified by MALDI-TOF/TOF mass spectrometry. Gene ontology analysis showed that those proteins participated in several important biological processes and exhibited diverse molecular functions. Importantly, four proteins (heterogeneous nuclear ribonucleoprotein H1 (H), isoform CRA_b, heterogeneous nuclear ribonucleoprotein A2/B1, isoform CRA_a, ubiquitin carboxyl-terminal hydrolase isozyme L1 and cathepsin D) acting as either oncoproteins or tumor suppressors draw our special attention. Finally, the effect of β-asarone on these four genes was confirmed at transcriptional level by semi-quantitative RT-PCR. Collectively, a variety of proteins affected by β-asarone were identified by 2-DE coupled with MALDI-TOF/TOF MS/MS analysis. Four potential protein targets were proposed, which will enable a better understanding of the anti-tumor activity of β-asarone.

1. Introduction

Malignant gliomas are the most common type of primary malignant brain tumor, accounting for approximately 80% of the 138,054 patients in the United States in 2010 and with 17,000 new cases diagnosed per year [1,2]. Glioblastoma, accounting for 82% of cases of malignant gliomas, is the most malignant form and characterized by rapid growth, highly invasiveness and enhanced angiogenesis [3]. Even with the latest combined treatment based on radiotherapy and temozolomide, which doubles the 2-year survival rate to 27%, the overall prognosis of patients with glioblastoma remains extremely poor [3]. Moreover, the blood–brain barrier (BBB), drug resistance and toxicity also hampered the effects of present therapies [4]. Therefore, the continued intensive investigation of new and innovative therapeutic options is critically required.

Rhizoma acori graminei (RAG), the dry rhizome of acorus gramineus solander (Araceae), has been used as a traditional oriental medicine for more than hundreds of years. In traditional Chinese medicine (TCM), RAG is mainly used for central nervous system (CNS) disorders. Previous investigations mainly focused on its effect and mechanisms on ameliorating learning and memory deficits and improving the cognitive function [5,6]. In addition, RAG is also frequently included in the formulations for treatment of brain tumor. However, the exact mechanisms have not been directly elucidated. Beta-asarone (1-propenyl-2,4,5-methoxybenzol) as shown in Fig. 1A is the major component in the volatile oil of RAG. Previous study demonstrated that β-asarone is the compound with the highest concentration detectable in the rat brain tissue after oral administration of the RAG extract [7]. Moreover, β-asarone changed the ultrastructure and improved the permeability of BBB [8]. It is also reported that β-asarone could inhibit the function

Abbreviations: RAG, rhizoma acori graminei; BBB, blood–brain barrier; SRB, sulforhodamine B; 2-DE, Two-dimensional gel electrophoresis; GO, gene ontology; hnRNP H1 isoform CRA_b, heterogeneous nuclear ribonucleoprotein H1 (H), isoform CRA_b; hnRNP A2/B1 isoform CRA_a, heterogeneous nuclear ribonucleoprotein A2/B1, isoform CRA_a; UCH L1, ubiquitin carboxyl-terminal hydrolase isozyme L1.

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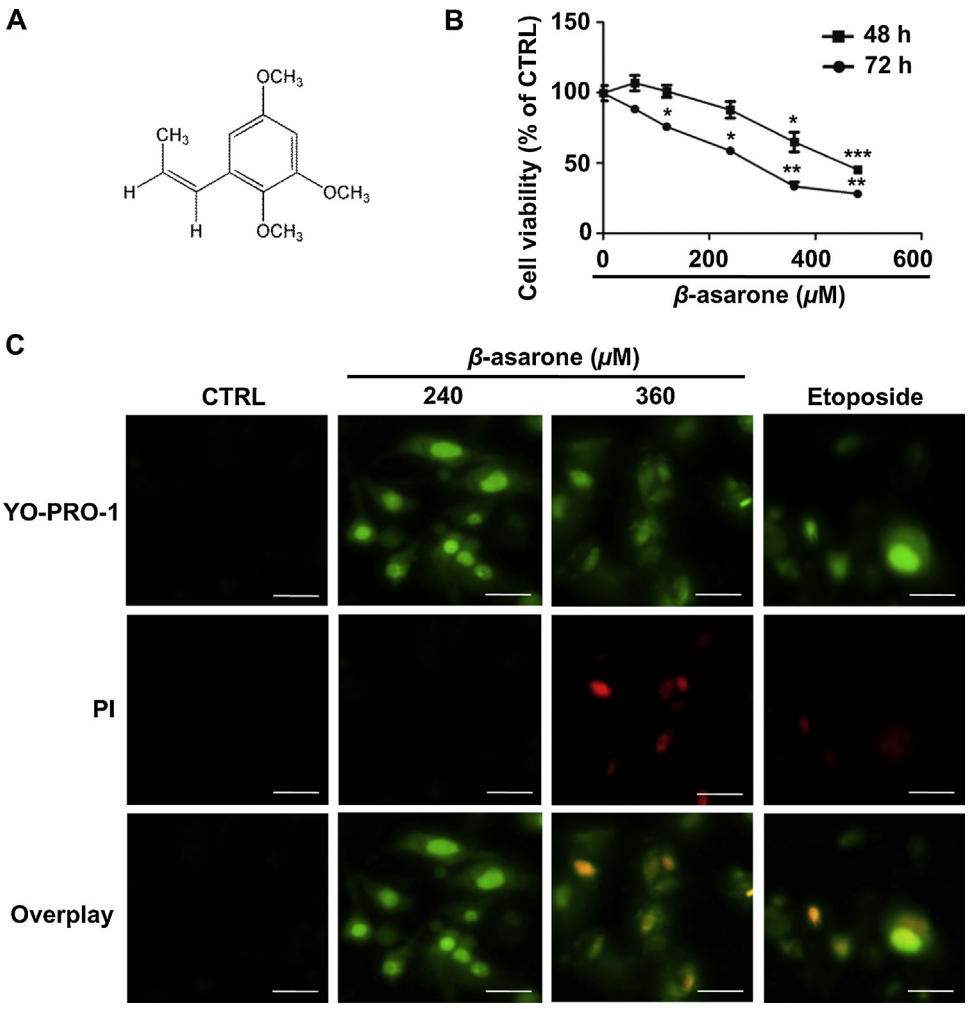

Fig. 1. Cytotoxic effect of β-asarone on U251 cells. (A) Chemical structure of β-asarone. (B) Effect of β-asarone on the cell viability of U251 cells. Cells were treated with vehicle or β-asarone for 48 or 72 h and the cell viability was determined by SRB assay described in the materials and method section. Values represent mean ± SD ($n = 6$). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. (C) YO-PRO-1 and PI dual staining. YO-PRO-1 shows numbers of apoptotic cells, and PI nuclei represent the necrotic cells. Scale of bar is 100 μm.

and expression of P-glycoprotein, which plays an important role in the multidrug resistance [9]. Therefore, β-asarone is believed to be the active compound for the effect of RAG on CNS disorders. Recent studies indicated that β-asarone exhibited anti-tumor effect against colorectal cancer by inducing apoptosis and senescence [10,11]. However, little is known about its inhibitory effect on brain tumor and the precise mechanism also remains unclear.

In the present study, the cytotoxic effect of β-asarone was first determined in human glioblastoma U251 cells. To comprehensively elucidate the underlying molecular mechanisms, a proteomic strategy was applied to characterize the key protein targets of β-asarone. The proteomic profiles of U251 cells treated with β-asarone and vehicle were compared and differentially expressed proteins were identified by MALDI-TOF mass spectrometry and annotated by Gene Ontology, and finally, the key protein targets were verified by confirmation of their expression level.

2. Materials and methods

2.1. Chemicals

Beta-Asarone was purchased from Dalian Meilun Biological Technology Co., Ltd. (Dalian, China) with purity more than 98%. Other chemicals were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA) unless indicated otherwise.

2.2. Cell culture

Human glioblastoma U251 cells were obtained from the American type cell culture collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Invitrogen) at 37 °C in a humidified 5% CO_2 atmosphere.

2.3. Measurement of cell viability

Cell viability was evaluated by sulforhodamine B (SRB) assay [12]. Briefly, at the end of drug treatment, cells were fixed with 50% (w/v) trichloroacetic acid (50 μL per well) and incubated at 4 °C for 1 h. The cells were then stained by exposure to 100 μL of SRB solution [0.4% SRB (w/v) in 1% acetic acid (v/v)] for 15 min, after which the plates were washed with 1% acetic acid to remove any unbound dye. Bound dye was solubilized with150 μL of 10 mM Tris base (pH 10.5). The plates were measured using a multi well spectrophotometer microplate reader (Biotek, Winooski, VT, USA) at a