J Ginseng Res 40 (2016) 278–284

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Proteomic analysis reveals that the protective effects of ginsenoside Rb1 are associated with the actin cytoskeleton in β-amyloid-treated neuronal cells

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ARTICLE INFO

Article history:
Received 28 August 2015
Received in Revised form
16 September 2015
Accepted 22 September 2015
Available online 1 October 2015

Keywords:
actin skeleton
Alzheimer's disease
β-amyloid
ginsenoside Rb1
mass spectrometry

ABSTRACT

Background: The ginsenoside Rb1 (Rb1) is the most abundant compound in the root of *Panax ginseng*. Recent studies have shown that Rb1 has a neuroprotective effect. However, the mechanisms underlying this effect are still unknown.

Methods: We used stable isotope labeling with amino acids in cell culture, combined with quantitative mass spectrometry, to explore a potential protective mechanism of Rb1 in β-amyloid-treated neuronal cells.

Results: A total of 1,231 proteins were commonly identified from three replicate experiments. Among these, 40 proteins were significantly changed in response to Rb1 pretreatment in β-amyloid-treated neuronal cells. Analysis of the functional enrichments and protein interactions of altered proteins revealed that actin cytoskeleton proteins might be linked to the regulatory mechanisms of Rb1. The CAP1, CAPZB, TOMM40, and DSTN proteins showed potential as molecular target proteins for the functional contribution of Rb1 in Alzheimer's disease (AD).

Conclusion: Our proteomic data may provide new insights into the protective mechanisms of Rb1 in AD.
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1. Introduction

The root of *Panax ginseng* (Ginseng) has been used in traditional oriental medicine to improve health for more than a thousand years in Asia. A number of studies have reported the neuroprotective effects of ginseng [1]. Cognitive behavior in patients with Alzheimer's disease (AD) was improved by ginseng powder [2]. Ginseng extract prevented the development of locomotion deficits in patients with Parkinson's disease [3].

The main bioactive components of ginseng are known as ginsenosides, which have been identified in > 30 species [4]. It has been reported that neuroprotective effects on central nervous system disorders and neuronal diseases can be attributed to the ginsenosides [5]. The effects of ginsenosides have been shown via increased cell survival, extension of neurite growth, and neuronal rescues both *in vivo* and *in vitro* [1]. Of these, ginsenoside Rb1 (Rb1) has been reported to be the primary ginsenoside responsible for the neuroprotective effects of neurodegenerative diseases [6]. Hippocampal neurons were protected by Rb1 against either ischemia or glutamate-induced neuronal diseases [7]. Recently, several studies have reported the protective effects of Rb1 against AD. Rb1 improved AD by increasing brain-derived neurotrophic factor and decreasing Tau protein [8] and protected neuronal cells from injury with β-amyloid (Aβ) treatment [9,10]. Additionally, Rb1 demonstrated anti-neuroinflammation effects in a rat model of AD [11].

In the past decade, many studies using state-of-the-art technologies have tried to understand the molecular mechanisms and

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http://dx.doi.org/10.1016/j.jgr.2015.09.004

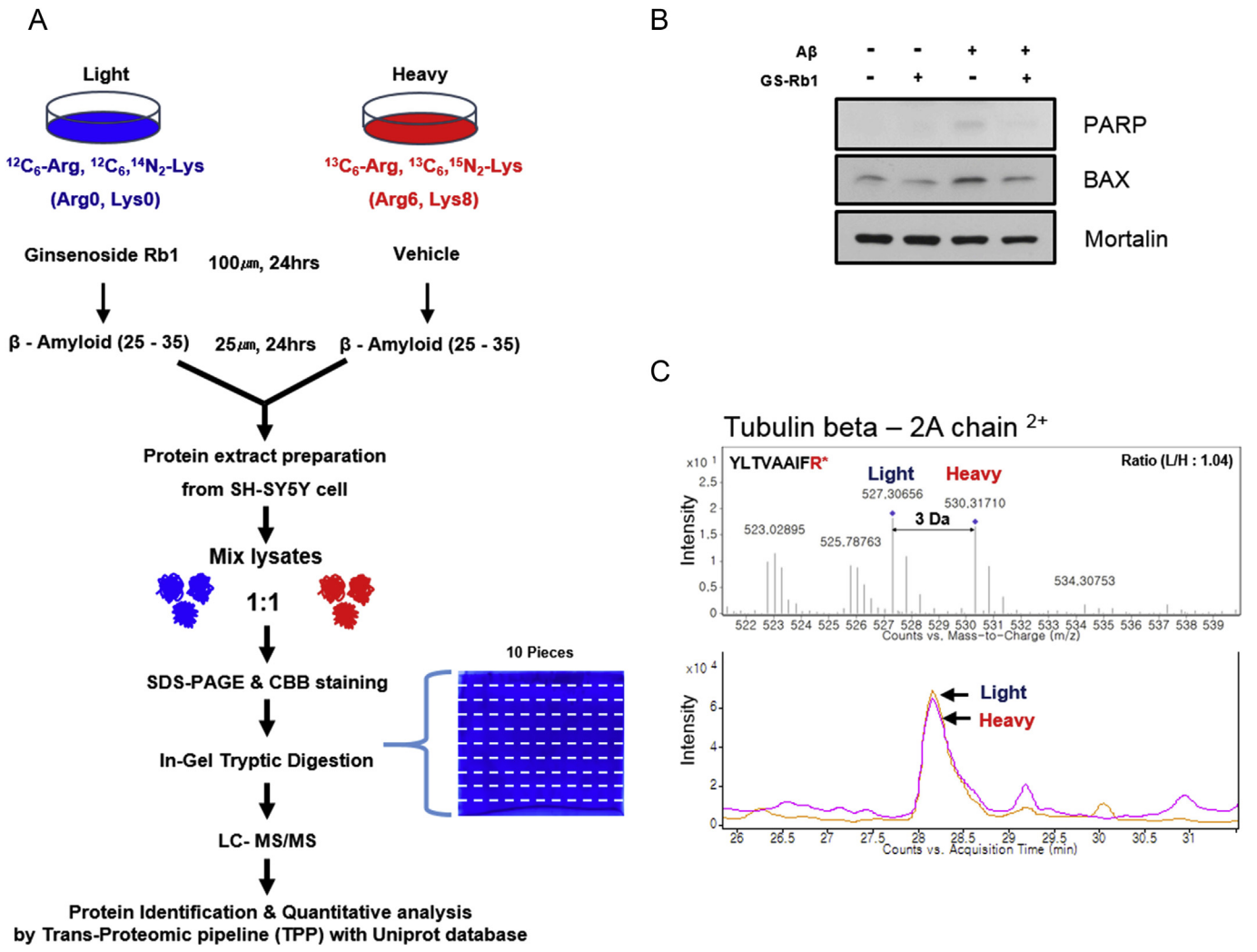

Fig. 1. SILAC analysis of pretreatment with Rb1 in Aβ-induced neurocytotoxicity. (A) An overview of SILAC experiments. Cells were pretreated with or without 100μM Rb1 for 24 h and then exposed to 25μM Aβ for 24 h. Each cell was lysed, and equal amounts of proteins were combined and then separated by SDS-PAGE. The gel lane was divided into 10 regions and analyzed using nano-LC MS/MS as described in the Materials and methods section. (B) Immunoblot analysis of Rb1 during Aβ exposure in SH-SY5Y cells. Decreased PARP-1 cleavage and Bax were observed with Rb1 pretreatment. (C). Paired peptides of a tubulin beta-2A chain showed an approximate ratio of 1:1. SH-SY5Y cells were cultured in light media containing $^{12}C_6$-Arg and $^{12}C_6$, $^{14}N_2$-Lys or heavy media containing $^{13}C_6$-Arg and $^{13}C_6$, $^{15}N_2$-Lys. Equal amounts of protein concentration were combined at a 1:1 ratio and were identified and quantified by nano-LC MS/MS. Aβ, β-amyloid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SILAC, stable isotope labeling with amino acids in cell culture.

to find biomarkers for the early diagnosis and treatment of AD [12]. In particular, proteomic studies, which provide powerful tools to identify the dynamic expression of proteins in biological samples, have been used to identify the molecular pathways involved in neuropathogenesis. From these studies, a number of potential target proteins have been identified for AD [13–15]. Recently, these proteomic studies have attempted to investigate the molecular effects of ginsenosides in cancer, smooth muscle cells, and diabetes [16–18]. However, even though a number of studies examining the protective effects of Rb1 are ongoing, our understanding of the regulatory mechanisms of Rb1 in AD is still lacking.

We performed a mass spectrometry (MS)-based proteomics experiment using stable isotope labeling with amino acids in cell culture (SILAC) to identify any proteins that are significantly altered by the neuroprotective effects of Rb1 in Aβ-treated neuronal cells. By following this approach, our data provide several new candidate proteins involved in the protective mechanisms of Rb1 and offer new insights into the potential molecular mechanisms of Rb1 in AD.

2. Materials and methods

2.1. SILAC

SILAC experiments were carried out as previously described [19]. In brief, SH-SY5Y cells were grown for at least five cell divisions in either "light media" containing $^{12}C_6$-Arg and $^{12}C_6$, $^{14}N_2$-Lys or "heavy media" containing $^{13}C_6$-Arg and $^{13}C_6$, $^{15}N_2$-Lys supplemented with 10% dialyzed fetal bovine serum (Invitrogen, New York, NY, USA), 50 IU/mL penicillin, and 50 mg/mL streptomycin. The labeled cells were pretreated with (light media) or without (heavy media) 100μM Rb1 for 24 h and then exposed to 25μM $A\beta_{25-35}$ (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. The cells were then lysed in buffer containing 1% Triton X-100, 150mM NaCl, 1mM EDTA, 50mM Tris–HCl (pH 8.0), 1mM sodium orthovanadate, 5mM NaF, 5mM sodium pyrophosphate, 1mM phenylmethylsulfonyl fluoride (PMSF), aprotinin (1.5 μg/mL), antipain (10 μg/mL), leupeptin (10 μg/mL), and benzamidine (0.1 mg/mL). The lysates were

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