J Ginseng Res xxx (2017) 1–9

Contents lists available at ScienceDirect

Journal of Ginseng Research

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

Research article

Photoaging protective effects of BIOGF1K, a compound-K-rich fraction prepared from *Panax ginseng*

Yo Han Hong [1,☆], Donghyun Kim [2,☆], Kyeonghwan Hwang [2], Sulgi Yoo [1], Sang Yun Han [1], Seonggu Jeong [1], Eunji Kim [1], Deok Jeong [1], Keejung Yoon [1], Sunggyu Kim [3], Junseong Park [2,**], Jae Youl Cho [1,*]

[1] *Department of Genetic Engineering, Sungkyunkwan University, Suwon, South Korea*
[2] *Amorepacific Research and Development Unit, Yongin, South Korea*
[3] *Research and Business Foundation, Sungkyunkwan University, Suwon, South Korea*

ARTICLE INFO

Article history:
Received 22 November 2016
Received in Revised form
25 December 2016
Accepted 2 January 2017
Available online xxx

Keywords:
anti-aging
BIOGF1K
Panax ginseng
photoaging
UVB irradiation

ABSTRACT

Background: BIOGF1K, a compound-K-rich fraction, has been shown to display anti-inflammatory activity. Although *Panax ginseng* is widely used for the prevention of photoaging events induced by UVB irradiation, the effect of BIOGF1K on photoaging has not yet been examined. In this study, we investigated the effects of BIOGF1K on UVB-induced photoaging events.

Methods: We analyzed the ability of BIOGF1K to prevent UVB-induced apoptosis, enhance matrix metalloproteinase (MMP) expression, upregulate anti-inflammatory activity, reduce sirtuin 1 expression, and melanin production using reverse transcription-polymerase chain reaction, melanin content assay, tyrosinase assay, and flow cytometry. We also evaluated the effects of BIOGF1K on the activator protein-1 signaling pathway, which plays an important role in photoaging, by immunoblot analysis and luciferase reporter gene assays.

Results: Treatment of UVB-irradiated NIH3T3 fibroblasts with BIOGF1K prevented UVB-induced cell death, inhibited apoptosis, suppressed morphological changes, reduced melanin secretion, restored the levels of type I procollagen and sirtuin 1, and prevented mRNA upregulation of MMP-1, MMP-2, and cyclo-oxygenase-2; these effects all occurred in a dose-dependent manner. In addition, BIOGF1K markedly reduced activator-protein-1-mediated luciferase activity and decreased the activity of mitogen-activated protein kinases (extracellular response kinase, p38, and C-Jun N-terminal kinase).

Conclusion: Our results strongly suggest that BIOGF1K has anti-photoaging activity and that BIOGF1K could be used in anti-aging cosmeceutical preparations.

1. Introduction

The skin is an important barrier that protects the body from external damage. A major cause of skin injury is UVB light. UVB emits light with wavelengths ranging from 280 nm to 320 nm. UVB irradiation of the skin results in various photoaging phenomena, such as induction of apoptosis, enhancement of matrix metalloproteinase (MMP) expression, upregulation of inflammation, and reduction of sirtuin (SIRT)1 expression [1–4]. In turn, activated MMPs contribute to extracellular matrix (ECM) degradation and synthesis inhibition, as well as that of collagen in connective tissues, thereby making a crucial contribution to photoaging [5]. Suppression of SIRT1 expression leads to upregulation of apoptosis and downregulation of cell survival [6,7]. These UVB-induced photoaging events are regulated by the activator protein (AP)-1 signaling pathway [8,9], which is in turn activated by mitogen-activated protein kinases (MAPKs), p38, extracellular response kinase (ERK), and c-Jun N-terminal kinase (JNK). Moreover, upon UVB irradiation, melanin is excessively synthesized by tyrosinase and secreted upon stimulation with α-melanocyte stimulating

* Corresponding author. Jae Youl Cho, Department of Genetic Engineering, Sungkyunkwan University, 2066 Seobu-ro, Suwon 16419, South Korea.
** Corresponding author. Junseong Park, Heritage Material Research Team, Amorepacific R&D Unit, 1920 Yonggu-daero, Yongin 17074, South Korea.
E-mail addresses: superbody@amorepacific.com (J. Park), jaecho@skku.edu (J.Y. Cho).
☆ These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jgr.2017.01.002
p1226-8453 e2093-4947/$ – see front matter

hormone (MSH) from epidermal melanocytes [10]. Melanin synthesis has also been shown to be predominantly regulated by the AP-1 signaling pathway [11].

The root of Korean ginseng (*Panax ginseng*) has been prescribed as an herbal medicine in East Asia. In addition to its anti-inflammatory and anticancer effects, this root has also been used as a cosmetic biomaterial due to its whitening, moisturizing, anti-wrinkle, and antiaging effects [12–14]. We recently prepared a fraction containing a high concentration of compound K, BIOGF1K, and found that it displayed antioxidative and anti-inflammatory activities [15]. To determine whether this fraction could have additional applications in various fields, we aimed to test whether BIOGF1K protected cells from UVB-induced photoaging events. To this end, we investigated BIOGF1K effects on UVB irradiation-induced apoptosis, morphological changes, melanin production, enzyme downregulation, inflammatory gene expression, and AP-1 signaling.

2. Materials and methods

2.1. Materials

Phorbol-12-myristate-13 acetate (PMA) and (3-4-5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The luciferase construct harboring AP-1 and collagen (Col)1A1 promoter binding sites was used as reported earlier [16,17]. TRIzol reagent was purchased from Molecular Research Center (Montgomery, OH, USA). Fetal bovine serum and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (Grand Island, NY, USA). The cell lines used in the present experiments (NIH3T3, HEK293, and B16F10 cells) were obtained from American Type Culture Collection (Rockville, MD, USA). All other chemicals were obtained from Sigma Chemical Co. Total and phosphospecific antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Plasmid constructs driving the expression of Smad3 (mothers against decapentaplegic homolog 3) were used as reported previously [18].

2.2. Cell culture

Mouse embryonic fibroblast NIH3T3 and mouse melanoma B16F10 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) in a CO_2 incubator at 37°C. For experiments, cells were seeded in six-well plates at 10^6 cells/well with fresh complete culture medium [19].

2.3. Preparation of BIOGF1K

BIOGF1K, a compound-K-rich fraction, was prepared as described previously [20].

2.4. Drug treatment

A stock solution of BIOGF1K was prepared in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL. Target concentrations (15 or microgram/ml and 30 μg/mL) were achieved by dilution with culture medium [21].

2.5. Cell viability assay

NIH3T3 cells were seeded onto 96-well plates at 10^5 cells/well with fresh complete culture medium. To test the cytotoxicity of BIOGF1K alone, cells were treated with 7.5, 15, or 30 μg/mL BIOGF1K. To test the effect of BIOGF1K on UVB-induced toxicity, cells were irradiated with 30 mJ/cm^2 UVB and then cultured in complete culture medium with 15 or 30 μg/mL BIOGF1K for a further 24 h. Cell viability was determined with a conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [22].

2.6. UVB irradiation

Cells were irradiated in six-well plates using a UVB lamp (Bio-Link BLX-312; Vilber Lourmat, Collégien, France) with an emission wavelength peak of 312 nm. Before UVB irradiation, culture medium was replaced with 1 mL phosphate-buffered saline (PBS) per well. After removing the plate lid, cells were irradiated at 30 mJ/cm^2. After UVB irradiation, PBS was replaced with complete culture medium with the appropriate compound treatments prior to harvesting [3].

2.7. HPLC analysis

The concentrations of compound K in BIOGF1K were quantified by HPLC as described previously [23,24].

2.8. Fluorescence-activated cell sorting

Apoptosis was analyzed by flow cytometry after different cell treatments. Cells were treated with or without 15 or 30 μg/mL BIOGF1K after UVB irradiation (30 mJ/cm^2) or subjected to a control treatment. For staining, cells were washed twice with cold PBS and resuspended in 1× binding buffer at a concentration of 10^6 cells/mL. Next, 100 μL of suspension (10^5 cells) was transferred to e tubes, 10 μL propidium iodide, and 5 μL fluorescein isothiocyanate–Annexin V was added, and cells were incubated for 15 min at room temperature in the dark. Finally, 400 μL 1× binding buffer was added and fluorescence was assessed using a Guava easyCyte flow cytometer (Millipore, Billerica, MA, USA) [25].

2.9. Plasmid transfection and luciferase reporter gene assay

For the luciferase reporter gene assay, HEK293 cells (10^5 cells/well in 24-well plates) were transfected with 0.8 μg/mL plasmids driving the expression of β-galactosidase, AP-1-Luc, Col1A1-Luc, and FLAG-smad3. Cells were transfected using the polyethyleneimine method and then incubated for 24 h. Finally, HEK293 cells ware treated with 15 or 30 μg/mL BIOGF1K and 100 nM PMA for a further 24 h [15].

2.10. Analysis of mRNA levels by reverse transcription-polymerase chain reaction

To quantify cytokine mRNA expression levels, NIH3T3 cells were treated with or without 15 or 30 μg/mL BIOGF1K after UVB irradiation (30 mJ/cm^2). Total RNA was then isolated with TRIzol reagent. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously [26]. Primers used in this study are listed in Table 1.

2.11. Immunoblotting

Total lysates prepared from NIH3T3 cells were subjected to western blot analysis of the total and phospho-forms of JNK, ERK, p38, IκBα, MAPK/ERK kinase (MEK)1/2, MAPK kinase (MKK)3/6, and β-actin. Immunoreactive bands were visualized as described previously [27].

Please cite this article in press as: Hong YH, et al., Photoaging protective effects of BIOGF1K, a compound-K-rich fraction prepared from *Panax ginseng*, Journal of Ginseng Research (2017), http://dx.doi.org/10.1016/j.jgr.2017.01.002