Biomedicine & Pharmacotherapy 82 (2016) 15–19

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Geranylgeranylacetone induces apoptosis via the intrinsic pathway in human melanoma cells

Ah Reum Jo[a], Hyo-Soon Jeong[a], Myo-Kyoung Kim[b], Hye-Young Yun[a], Kwang Jin Baek[a], Nyoun Soo Kwon[a], Dong-Seok Kim[a,*]

[a] Department of Biochemistry, Chung-Ang University College of Medicine, 84 Heukseok-ro Dongjak-gu, Seoul 06974, Republic of Korea
[b] Thomas J. Long School of Pharmacy, University of the Pacific, 3601 Pacific Avenue, Stockton, CA 95211, USA

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form 25 April 2016
Accepted 25 April 2016

Keywords:
Apoptosis
Caspase
Geranylgeranylacetone
Melanoma
Skin cancer

ABSTRACT

The aim of this study was to test the anti-cancer effects of geranylgeranylacetone (GGA), an isoprenoid compound, on human melanoma cells. Human melanoma cell lines G361, SK-MEL-2, and SK-MEL-5 were treated with GGA at various doses (1–100 μM). Cell viability was measured by crystal violet assay. Western blot analysis was adopted to detect marker proteins of apoptosis. GGA significantly reduced the viability of G361, SK-MEL-2, and SK-MEL-5 human melanoma cells at concentrations above 10 μM. Western blot analysis showed the phosphorylation of p38 MAPK and c-Jun N-terminal kinase (JNK) after GGA treatment, as well as activation of caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage. GGA also induced p53 and Bax expression, but did not affect expression of Bcl-2 and MITF. These findings suggest that GGA induces apoptosis through the intrinsic pathway. Accordingly, GGA should be considered for further development as a potential agent for melanoma.

1. Introduction

The incidence of melanoma, the most fatal form of skin cancer, has increased significantly over the past 20 years [1]. Melanoma, which occurs from normal melanocytes, is an aggressive tumor with frequent metastases and high mortality that is treated primarily with surgery, chemotherapy, and radiotherapy [2]. Although the surgical resection of tumors can be successful at earlier stages, melanoma is fatal and intractable in cases of late diagnosis [3]. Accordingly, there is a growing interest in drugs that would have the ability to inhibit proliferation and initiate the apoptotic pathway [4], and research and novel treatments are needed.

When cells are under physiological and pathological stress, they are programmed to commit suicide through processes called apoptosis [5]. The two apoptotic pathways are commonly known as the intrinsic and extrinsic pathways. The cell death receptor-mediated extrinsic pathway initiates activation of caspase-8 and its downstream caspase-3 [6]. The intrinsic pathway is intimately related to mitochondrial membrane permeabilization through regulation of the Bcl-2 family, which is initiated by cellular stress such as UV radiation, chemotherapeutic agents, irradiation, and oxidative stress [7,8]. There are two sub-families in the Bcl-2 family. Pro-apoptotic proteins such as Bax and Bad help activate the cellular apoptotic pathway by increasing mitochondrial membrane permeabilization, whereas anti-apoptotic proteins such as Bcl-2 and Bcl-xl prevent this action. The apoptosome subsequently formed by cytochrome c, Apaf-1, and caspase-9 induces the activation of caspase-3 and ultimately leads to apoptosis [7,9,10]. In addition, members of the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinase (ERK), p38 MAPK, and c-jun N-terminal kinase (JNK), play essential roles in regulating cell survival and apoptosis [11]. According to several studies, activation of p38 MAPK is related to skin cell death in response to UV [12] and stress-induced apoptosis [13]. This association suggests that the MAPK pathway is largely involved in controlling skin cell fates under various environmental conditions.

Geranylgeranylacetone (GGA), an isoprenoid compound, has been used as an anti-ulcer drug since 1984 in Japan with few adverse effects [14]. Previous studies have reported that GGA acts as an inducer of Heat shock protein 70 [15,16]. Heat shock proteins (HSPs), heat-inducible cytoprotective proteins, accumulate first in

Abbreviations: ERK, extracellular signal-regulated kinase; GGA, geranylgeranylacetone; HSP70, heat shock protein 70; JNK, c-jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MITF, microphthalmia-associated transcription factor; PARP, poly(ADP-ribose) polymerase.

* Corresponding author.
E-mail address: ds_kim@cau.ac.kr (D.-S. Kim).

http://dx.doi.org/10.1016/j.biopha.2016.04.051
0753-3322/

cells after thermal stress [16]. In particular, HSP70 is well known as an endogenous factor for cell survival [17]. Overexpression of HSP70 can protect cells from stress-induced apoptosis and interfere with the release of cytochrome c [18]. Therefore, enhancing HSP70 expression with GGA has a protective effect against stress ulcer formation, and in several human injury models [19,20]. At the same time, GGA suppresses cell survival and induces apoptosis by modulating small G-protein activation in leukemia cells [21]. Furthermore, GGA has anti-invasion effects on ovarian carcinoma cells and breast cancer cells [22,23].

Although GGA has many biological activities, there has been no study of the effects of GGA on human melanoma cells. Therefore, the purpose of this study was to investigate the effect of GGA on melanoma cell death. The effect of GGA on signal transduction pathways was also examined.

2. Materials and methods

2.1. Materials

GGA was purchased from Sigma Aldrich (St. Louis, MO, USA). Antibodies recognizing HSP70/HSC70 (W27, sc-24), caspase-8 (sc-7890), caspase-9 (sc-8355), Bax (sc-526), Bcl-2 (sc-7382), actin (I-19), poly(ADP-ribose) polymerase (PARP, sc-7150), and p53 (sc-126) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and anti-MITF antibody (Ab-3, C5 + D5, MS-773-P0) was obtained from NeoMarkers (Fremont, CA, USA). Antibodies against cleaved caspase-3 (CST-9661), phospho-p38 MAP kinase (CST-9211), p38 MAP kinase (CST-9212), phospho-SAPK/JNK (CST-9251), and SAPK/JNK (CST-9258), as well as SP600125 were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). SB203580 was purchased from Calbiochem (Darmstadt, Germany).

2.2. Cell culture

The lightly pigmented human melanoma cell line G361 (ATCC, Rockville, MD, USA) and human melanoma cell lines SK-MEL-2 and SK-MEL-5 were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin (10,000 U/ml and 10,000 μg/ml, respectively) under 5% CO_2 at 37 °C.

2.3. Cell viability

Cell viabilities were measured by crystal violet assay. Cells were seeded onto 24-well plates at 5×10^4 cells per well, serum-starved for 24 h, and treated with GGA at various doses for 24 h. After removing the culture media, cells were stained with 0.1% crystal violet in 10% ethanol for 10 min at room temperature and then rinsed four times with distilled water. The crystal violet retained by adherent cells was extracted with 95% ethanol, and absorbance was determined in lysates at 590 nm by an ELISA reader (VERSAMax; Molecular Devices, Sunnyvale, CA, USA).

2.4. Western blot analysis

G361 cells were grown in 100-mm culture dishes. After serum starvation, cells were treated with GGA for 24 h and then lysed in cell lysis buffer [62.5 mM Tris–HCl (pH 6.8), 5% β-mercaptoethanol, 2% SDS, 2 mM phenylmethylsulfonyl fluoride, protease inhibitors (Roche, Mannheim, Germany), 50 mM NaF, 1 mM Na_3VO_4, and 10 mM EDTA]. Next, 20 μg of protein per lane were separated by SDS polyacrylamide gel electrophoresis, blotted onto PVDF membranes, and then blocked with 5% non-fat dried milk in Tris-buffered saline containing 0.5% Tween 20. Blots were incubated with primary antibodies at 4 °C overnight and then further incubated with suitable horseradish peroxidase-conjugated secondary antibodies. Bound antibody was identified using an enhanced chemiluminescence (ECL) solution. All images of the blotted membranes were obtained using an LAS-1000 luminoimage analyzer (Fuji Film, Tokyo, Japan).

2.5. Statistical analysis

The student's *t*-test was used to evaluate differences between groups, and *P* values <0.05 were considered statistically significant.

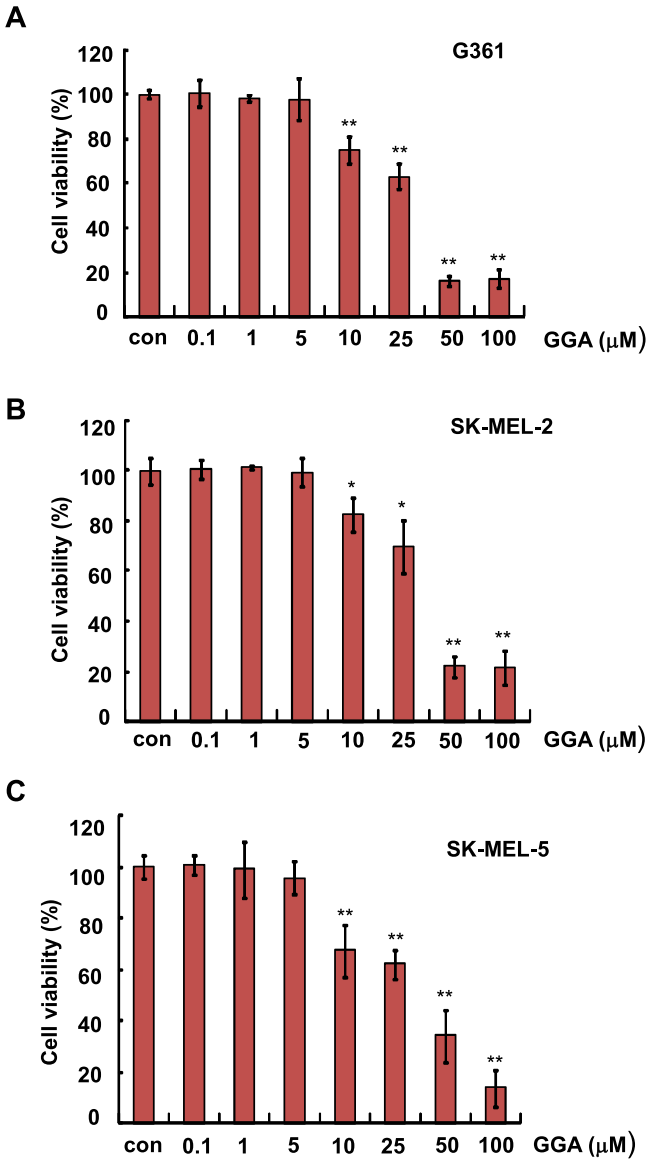

Fig. 1. Effect of GGA on cell viability of human melanoma cells. (A) G361 cells, (B) SK-MEL-2 cells, and (C) SK-MEL-5 cells were treated with GGA at various doses for 24 h. Cell viability was measured by crystal violet assay. The data represent the mean ± S.D. from three independent experiments. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with the control.