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[6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells

Hyun Sook Lee[a], Eun Young Seo[b], Nam E Kang[c], Woo Kyung Kim[b,*]

[a]Department of Sports Sciences, Seoul Sports Graduate University, Seoul 150-034, South Korea
[b]Department of Food Sciences and Nutrition, College of Natural Sciences, Dankook University, Youngsan-gu, Seoul 140-714, South Korea
[c]Major of Food and Nutrition, School of Food Science, Eulji University, Sungnam 461-250, South Korea

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Abstract

Gingerol (*Zingiber officinale Roscoe, Zingiberaceae*) is one of the most frequently and heavily consumed dietary condiments throughout the world. The oleoresin from rhizomes of ginger contains [6]-gingerol (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-decanone) and its homologs which are pungent ingredients that have been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, antihepatotoxic and cardiotonic effects. However, the effects of [6]-gingerol on metastatic processes in breast cancer cells are not currently well known. Therefore, in this study, we examined the effects of [6]-gingerol on adhesion, invasion, motility, activity and the amount of MMP-2 or -9 in the MDA-MB-231 human breast cancer cell line. We cultured MDA-MB-231 cells in the presence of various concentrations of [6]-gingerol (0, 2.5, 5 and 10 μM). [6]-Gingerol had no effect on cell adhesion up to 5 μM, but resulted in a 16% reduction at 10 μM. Treatment of MDA-MB-231 cells with increasing concentrations of [6]-gingerol led to a concentration-dependent decrease in cell migration and motility. The activities of MMP-2 or MMP-9 in MDA-MB-231 cells were decreased by treatment with [6]-gingerol and occurred in a dose-dependent manner. The amount of MMP-2 protein was decreased in a dose-dependent manner, although there was no change in the MMP-9 protein levels following treatment with [6]-gingerol. MMP-2 and MMP-9 mRNA expression were decreased by [6]-gingerol treatment. In conclusion, we have shown that [6]-gingerol inhibits cell adhesion, invasion, motility and activities of MMP-2 and MMP-9 in MDA-MB-231 human breast cancer cell lines.

Keywords: [6]-Gingerol; Metastasis; MMP-2; MMP-9

1. Introduction

Numerous epidemiological, biological and clinical studies indicate a strong correlation between dietary factors and prevention of human cancers [1–3]. Therefore, chemoprevention of cancers by nutraceuticals and phytochemicals has become a flourishing field of research over the past decade [4–7].

Ginger rhizome (*Zingiber officinale*), commonly known as ginger, is utilized worldwide as a spice and a flavoring agent. It has a long history of both culinary and medicinal use [8]. Ginger contains pungent phenolic substances collectively known as gingerols. [6]-Gingerol (1,[4′-hydroxy-3′-methyoxyphenyl-5-hydroxy]-3-decanone), one of the major pungent elements of ginger, has been found to exhibit antioxidant activity as determined by inhibition of phospholipid peroxidation induced by the $FeCl_3$-ascorbate system [9] and confirmed in many in vitro and in vivo system [10,11]. Gingerol has also been found to inhibit platelet aggregation and formation of prostaglandin and leukotriene [12–14]. Ginger oil at a single oral dose of 33 mg/kg significantly suppressed severe chronic adjuvant arthritis in rats [15]. Ginger consumption ameliorated the pain and symptoms of rheumatic disorders [16,17] and lowered the platelet thromboxane production in humans [18].

Recently, the cancer chemopreventive potential of ginger has been determined by the inhibition of phobol ester-induced inhibition of Epstein–Barr virus activation I Raji cells [19] and suppression of azoxymethane-induced intestinal carcinogenesis in rats and mouse skin tumor [20,21].

* Corresponding author. Tel.: +82 2 709 2407; fax: +82 2 792 7960.
E-mail address: wkkim@dankook.ac.kr (W.K. Kim).

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Also, gingerol has been shown to exhibit anticancer activities through the induction of apoptosis [22,23].

Cancer metastasis consists of a complex cascade of events, which ultimately allow for tumor cell escape and seeding of ectopic environments [24]. For breast cancer cells to manifest their malignant potential, they must develop the ability to break through and dissolve extracellular matrix (ECM), particularly the delimitating basement membrane (BM). The degradation of the pericellular BM and ECM is catalyzed by the concerted action of several classes of ECM-degrading enzymes. One important class of ECM-degrading enzymes includes the matrix metalloproteinases (MMPs) [25]. MMPs have been implicated as possible mediators of invasion and metastasis in some cancers. Kim et al. [26] recently showed that [6]-gingerol inhibited the formation of lung metastases of B16F10 melanoma in an experimental mouse model. However, there is limited knowledge regarding the effect of [6]-gingerol in terms of metastasis and MMPs, by which it may exert its antitumor effects.

In this study, we investigated the effect of [6]-gingerol on tumor metastasis. Thus far, no studies have been undertaken to assess the effect of ginger on metastasis in breast cancer. Therefore, we examined the effect of [6]-gingerol against MDA-MB-231 human breast cancer cells.

2. Materials and methods

2.1. Materials and reagents

[6]-Gingerol was purchased from Biomol (USA) and was dissolved in ethanol and diluted in cell culture medium. MDA-MB-231 cells were purchased from American Type Culture Collection (Rockville, MD, USA). The following reagents and chemicals were obtained from the respective suppliers: Dulbecco's modified Eagle's medium/Nutrient Mixture Ham's F12 (DMEM/F12), streptomycin and penicillin (Gibco/BRL); RIA-grade bovine serum albumin (BSA) and transferrin (Sigma, St. Louis, MO, USA); antibodies for MMPs were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other reagents were obtained from Sigma.

2.2. Cell culture

The MDA-MB-231 human breast cancer cell line was maintained in DMEM/F12 containing 100 ml/L of fetal bovine serum (FBS) with 100,000 U/L of penicillin and 100 mg/L of streptomycin. To examine the effect of [6]-gingerol, cells were plated with DMEM/F12 containing 10% FBS. Before MDA-MB-231 human breast cancer cells were treated with [6]-gingerol, the cell monolayers were rinsed and starved of serum for 24 h, with DMEM/F12 supplemented with 5 mg/L transferrin, 1 g/L BSA and 5 μg/L selenium (serum-free medium, SFM). After serum starvation, fresh SFM with or without the indicated concentrations of [6]-gingerol was replaced. Viable cell numbers were estimated 24, 48 and 72 h after the cells were exposed to [6]-gingerol using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously [27]. In brief, the MTT solution (0.5 mg/ml) was added to the cells and incubated at 37°C to allow cleavage of the tetrazolium ring by mitochondrial dehydrogenases and formation of blue formazan crystal. After 3 h, the residual MTT was carefully removed and crystals were dissolved with isopropanol. The absorbance at 470 nm was measured by spectrophotometry.

2.3. Adhesion assay

Ninety-six-well plates were coated with fibronectin (BD, Bioscience, Massachusetts, USA, 20 μg/1 ml PBS) and incubated for 1 h at 37°C with 5% CO_2. Coated wells were washed twice with PBS and incubated for 1 h with SFM. The wells were rewashed with PBS and dried on a clean bench, then MDA-MB-231 cells (8×10^5 cells/well) suspended in the medium containing 0, 2.5, 5 and 10 μM [6]-gingerol were seeded into coated wells and incubated for 1 h at 37°C. Adherent cells were washed three times with PBS and reincubated in a medium containing 1 mg/ml MTT for 3 h at 37°C, and the absorbance was measured at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Experiments were performed independently three times.

2.4. Matrigel invasion assay

Wells of a matrigel chamber (BD, Bioscience) were filled with SFM and adapted at room temperature. MDA-MB-231 cells (1×10^6 cells/ml) resuspended in the medium containing 0, 2.5, 5 and 10 μM [6]-gingerol were carefully transferred into the upper chambers. Lower chambers were filled with 10% FBS medium to attract cells. Matrigel chambers were incubated for 12 h at 37°C with 5% CO_2. Then, the cells on the upper surfaces of the filters were removed by wiping with a paper. Filters were stained with Diff-Quik stain solution (Dade Behring, Newark, NJ, USA), and the cells on the lower surface of the filter were fixed onto a glass slide. Cells in five randomly selected microscopic fields (×400) of the lower slide were counted. Experiments were performed independently three times.

2.5. Wound healing migration assay

Wound-healing migration assay is based on the repopulation of wounded cultures. The cells were seeded into 12-well culture plates at 5×10^5 cells/ml and cultured in medium containing 10% FBS to near confluence of the cell monolayer. Confluent cell monolayers were incubated for 1 h with 1 μg/ml mytomicin C to stop cell proliferation. The monolayers were carefully wounded using a yellow pipette tip, and any cellular debris present was removed by washing with PBS. The wounded monolayers were then incubated for 48 h in SFM containing 0, 2.5, 5 and 10 μM [6]-gingerol. Photographs of the exact wound areas taken initially were again taken after 12, 24 and 48 h.