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Sasanquasaponin from *Camellia oleifera* Abel. induces cell cycle arrest and apoptosis in human breast cancer MCF-7 cells

Linyan Chen [a], Jing Chen [b], Hanhong Xu [a,*]

[a] Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, Guangzhou 510642, China
[b] Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine at Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China

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ABSTRACT

Sasanquasaponin (SQS) is a triterpenoid extracted from the Chinese medicinal plant *Camellia oleifera* Abel. Little knowledge about the role of SQS in the prevention and treatment of cancer is available. Recent studies have shown that SQS possesses potent anti-tumor activities in human leukemia Jurkat cells and human hepatoma HepG2 cells. However, research on the effects and mechanisms of SQS on the treatment of breast cancer, a major cause of mortality in women worldwide, is not available. In this work, the effects of SQS on cell growth characteristics, including proliferation, cell cycle progression, and apoptosis, in MCF-7 cells were investigated. SQS reduced the viability of MCF-7 cells, induced G1 phase arrest in the cell cycle, and triggered the apoptosis of MCF-7 cells. Stimulation of MCF-7 with SQS induced upregulation of p21 and downregulation of cyclin D1, which can cause G1 cell cycle arrest. Stimulation also induced activation of E2F1 and downregulation of p53, indicating that SQS induces cell cycle arrest and apoptosis via a p53-independent pathway; p53-independent apoptosis may be mediated by E2F1 activation. Our results demonstrate the potential application of SQS as an anti-breast cancer agent.

1. Introduction

Breast cancer is the most common cancer among women throughout the world [1,2] and one of the leading causes of death due to cancer [3]. Over one million people worldwide have been diagnosed with this deadly disease, which causes over 400,000 deaths annually [4]. Several drugs have been developed to treat breast cancer but therapy is commonly interrupted by drug resistance, a common occurrence [5–8]. Tamoxifen resistance is common in estrogen receptor-α-positive breast cancers [8], and tamoxifen has been reported to be effective in only one-third of all breast cancer patients [9]. Conventional therapeutic strategies, including surgery, radiation, and chemotherapy, produce significant side effects and their effectiveness is limited [10]. Thus, the development of anti-breast cancer drugs is an urgent matter. Epidemiological investigations and laboratory studies have indicated that compounds developed from natural sources play an important role in the prevention and treatment of many cancers [11,12].

Camellia oleifera Abel. has been widely cultivated in many parts of China. The seeds of this plant are used for oil production, during which large amounts of the byproduct, tea-seed cake, are produced and discarded as waste residues [13]. Tea-seed cake contains approximately 8% saponins [14] and about 10,000,000 tons of tea-seed cake is generated in China each year [15]. Utilization of sasanquasaponin (SQS) extracted from tea-seed cake may be a mutually beneficial undertaking from the medical and agricultural points of view.

SQS is a biologically active ingredient extracted from *C. oleifera* Abel [16]. The main structure of SQS (22-*O*-angeloylcamelliagenin C 3-*O*-[β-D-glucopyranosyl (1,2)]

Abbreviations: SQS, sasanquasaponin; HPLC, high-performance liquid chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; EtOAc, ethyl acetate; BuOH, *n*-butanol; DMEM, Dulbecco's modified Eagle's medium; NEAA, nonessential amino acids; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; PVDF, polyvinylidene difluoride.

* Corresponding author at: Department of Pesticides, College of Natural Resources and Environment, South China Agricultural University, 483 Wushan Road, Guangzhou 510642, China. Tel./fax: +86 20 85285127.
E-mail address: hhxu@scau.edu.cn (H. Xu).

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[β-D-glucopyranosyl (1,2)-α-L-arabinopyranosyl (1,3)]-β-D-glucopyranosiduronic acid, $C_{58}H_{92}O_{26}$, SQS) is a triterpenoid (Fig. 1A), similar to the structure of some ginseng saponins [17,18]. SQS has many pharmacological properties, including anti-inflammation, anti-hyperlipidemia, and anti-effusion [19]. Recent studies have shown that SQS can induce apoptosis in human leukemia Jurkat cells and hepatoma HepG2 cells [20,21]. However, research on the effects and mechanisms of SQS on the treatment of breast cancer, a major cause of mortality in women worldwide, is currently unavailable.

In the present study we determine whether or not SQS induces cell cycle arrest and apoptosis in the human breast cancer cell line MCF-7 and investigate the possible mechanisms by which these actions occur.

2. Materials and methods

2.1. Chemicals

SQS with a purity of 96% was extracted, isolated, purified and identified by the Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University. Extraction, isolation and purification of sasanquasaponin were performed according to the previously described method [22]. In brief, the defatted tea-seed cake of *C. oleifera* Abel. was broken into pieces, and extracted with boiling water three times. After filtration, the water extract was concentrated, and the concentrate was extracted with ethyl acetate (EtOAc) and *n*-butanol (BuOH) in turn. After that, the BuOH layer was evaporated, and the extract was fractionated by silica gel column chromatography to obtain some crude crystals. The crude crystals were repurified by preparative HPLC (eluation system: BuOH-water) to give SQS. Its purity was further determined by reversed-phase high-performance liquid chromatography (RP-HPLC) to be approximately 96%. SQS was dissolved in DMSO to make a 50 mM stock solution and stored at −20 °C. Each working solution was freshly prepared in the cell culture medium with a final DMSO concentration of less than 0.1%. All culture media and serum were obtained from GIBCO-BRL Life Technology, Inc. All other reagents and plastic wares were obtained from commercial sources.

2.2. Cell culture

MCF-7 human breast cancer cells were obtained from the Cell Bank of Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences. Cells were grown in DMEM supplemented with Glutamax (2 mM), NEAA (0.1 mM) and 10% fetal bovine serum in a humidified environment with 5% CO_2 at 37 °C. The medium was replaced at 2-day intervals. Subconfluent cells were routinely harvested with 0.05% trypsin–0.02% EDTA.

2.3. MTT assay

Cell growth was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. Following treatment of MCF-7 cells with SQS for 24 h, 20 μl of 5 mg/ml MTT was added to cell culture medium. After 4 h incubation at 37 °C, the culture medium was discarded and 0.1 ml

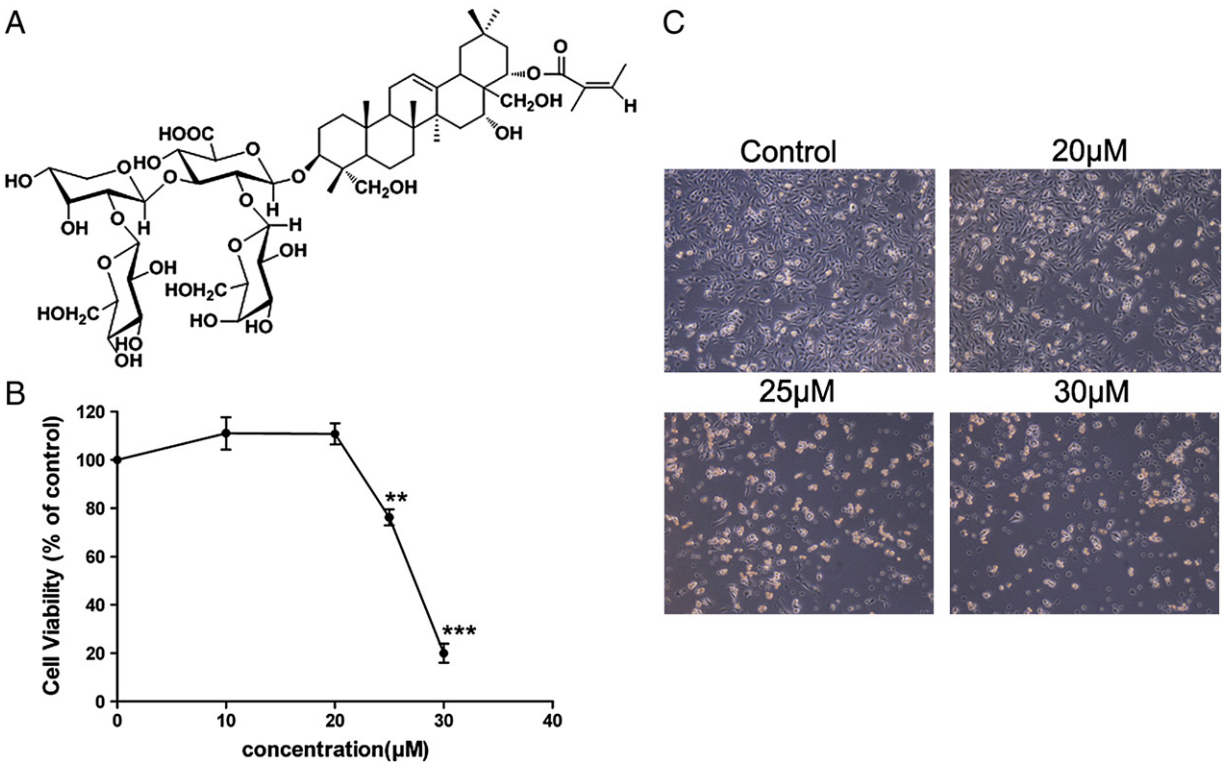

Fig. 1. SQS inhibits the growth of MCF-7 cells. (A) Chemical structure of SQS. (B) MCF-7 cells were treated with different concentrations of SQS for 24 h. Cell viability was determined by the MTT assay. (C) MCF-7 cells were treated with different concentrations of SQS for 24 h and observed under an inverted light microscope.