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Anti-proliferative effect of *Zea mays* L. cob extract on rat C6 glioma cells through regulation of glycolysis, mitochondrial ROS, and apoptosis

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ABSTRACT

Gliomas are one of the most common types of primary brain tumors, characterized by rapid proliferation and infiltration into normal brain tissue. Corncob is the most plentiful byproducts of *Zea mays* L., of which anticancer effect has not been reported. Therefore, we aimed to examine the anti-proliferative effect of a high-pressure hot-water extract of corncob on glioma cells and elucidated the underlying mechanism. The high-pressure hot-water corncob extract contained approximately 94.8 mg/g and 1.82 μg/g of total phenol and catechin, respectively. Glioma cell treated with different concentrations of high-pressure hot-water corncob extract was shown to be suppressed in growth during three days of culture. In parallel, corncob extract reduced the glioma cell viability and induced cell cycle arrest in G0/G1 phase by upregulating the expression level of cyclin-dependent kinase inhibitor p21. Decreased proliferation and viability in glioma cells treated with corncob extract can be attributed to reduced reactive oxygen species (ROS), antiapoptotic Bcl-2 protein, and a lactate transporter monocarboxylate transporter 1 of which levels are higher than those in normal cells. Based on its inhibitory effects on proliferation and viability of C6 glioma cells, a high-pressure hot-water corncob extract has the potential to be used for glioma treatment.

1. Introduction

One of the commonest brain tumors in adults is malignant glioma, which is characterized by rapid and invasive growth [1]. Due to its infiltration throughout the brain parenchyma, relying on surgery and chemotherapy to effectively remove these tumors is challenging; consequently, glioma is associated with high morbidity, high mortality, and an extremely poor prognosis. In addition, although several therapeutic agents are being used for glioma treatment [2,3], their efficacy has been shown to be usually poor due to high levels of drug resistance and the inability of drugs to cross the blood-brain barrier effectively [4]. Therefore, the development of therapeutic targets is increasingly needed to limit the rapid and invasive growth of malignant gliomas.

Reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl free radical (HO) are representative chemically reactive signal molecules, derived from aerobic metabolism [5,6], that have essential functions in living organisms including signaling growth, cell differentiation, and inflammatory responses [7]. Compared to their normal counterparts, cancer cells have been shown to have increased levels of ROS due to their high basal metabolic rate, which is associated with aberrant cancer cell growth and survival [7–9]. As elevated levels of ROS can increase the vulnerability of cancer cells to damage by prolonged ROS insults, one therapeutic approach has been to treat cancer cells with exogenous agents that induce ROS production [5,7,10]. Another approach has been to increase ROS scavenging, thereby interfering with H_2O_2 signaling and inhibiting cell growth [7,11].

Since most cancer cells exhibit increased glycolytic metabolism, which in turn generates ATP as the main source of their energy supply, many studies have investigated glycolytic metabolism of tumors as a means to inhibit cancer growth [12]. One approach has been to block tumor glycolysis by inhibiting the monocarboxylate transporters (MCTs), which control cancer-cell lactate export [13]. MCTs are a family of membrane proteins that includes 14 members. Among those are

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four MCTs (MCT1–MCT4) that are known to catalyze proton-linked bi-directional transport of lactate, pyruvate, and ketone bodies [14]. MCT1 is expressed in a great majority of tissues and cancer cell lines [15,16]. Most recently, MCT1 inhibition was reported to block tumor growth via lactate transport and glycolysis impairment, which in turn reduced cancer cell proliferation and tumor growth [13].

Along with cornhusk and corn silk, corncob is a byproduct of *Zea mays* L. (*Z. mays*). In agricultural production, yields of corncob and cornhusk are notably higher than that of corn silk [17,18]. Stigma maydis (corn silk) has been extensively studied and has been reported to have significant bioactivities including antitumor, antioxidant, antibacterial, antidepressant, anti-fatigue, and it can also prevent hyperglycemia [19–23]. Conversely, the bioactivities of corncob have not been widely studied, except for a study into its antioxidant properties [17].

Since rapid proliferation, infiltration, and recurrence are common in glioma, preventing glioma cell proliferation is an important therapeutic strategy in the treatment of this tumor. The present study was undertaken to study the anti-proliferative effects of a high-pressure hot-water corncob extract on rat C6 glioma cells, as an experimental model of glioma growth and infiltration [24].

2. Materials and methods

2.1. Preparation of corncob extract

Dried corncobs (50 g) were mixed with 500 mL distilled water. The extraction process was performed at 110 °C and 5.69 PSI. for 15 min in an autoclave (Wiseclave WAC-60, Daihan Scientific), followed by filtration with a 0.2 μm syringe filter (Minisart, Sartorius). The extract was then freeze-dried, and stored in the dark until use. For later use, the freeze-dried extract was reconstituted in distilled water to obtain a concentration of 3.3 mg/mL.

2.2. Measurement of total phenol and catechin contents in corncob extract

Total phenol and catechin contents were measured by using the Folin-Ciocalteu method and Vanillin assays, respectively, as described previously [25].

2.3. Cell culture, cell growth curves, and cell viability assay

Rat C6 glioma cells, PC12 pheochromocytoma, and noncancerous NIH/3T3 cells were obtained from ATCC. Cells were cultured in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gemcell) at 37 °C in a CO_2 incubator (Thermo Scientific; 95% air and 5% CO_2). To examine the effects of the corncob extract or cisplatin on cell growth, C6 glioma, PC12 or NIH/3T3 cells were plated at a density of 4000 cells per well in a 24-well plate. After incubation for 12 h, cells were treated with corncob or cisplatin at the indicated concentrations (0.33, 3.3, and 33 μg/mL for corncob; 0.5, 3.0, and 10.0 μg/mL for cisplatin) by using the culture medium as a vehicle. Cells were counted on days 0, 1, 2, and 3 by using a hemocytometer after staining with 0.4% (w/v) trypan blue dye (Thermo Fisher).

The effects of corncob extract and cisplatin on cell viability were examined by using the WST-1 assay kit (DoGenBio) as described previously [25]. Briefly, cells were seeded in 96-well plates, with an average of 10^4 cells/well, in 100 μL of media. Cells were incubated overnight, followed by and then treated in triplicate for 24 h at 37 °C in a 5% CO_2 incubator with corncob extract or cisplatin at indicated concentrations. Ten microliters of EZ cytox cell viability assay reagent was added to each well, followed by incubation for 2 h at 37 °C in a 5% CO_2 incubator. Finally, the optical density was measured at 450 nm using a microplate reader (Sunrise™, Tecan). All experiments were repeated at least three times.

2.4. Cell cycle analysis

Rat C6 glioma cells (10^4) were seeded into 60 mm plates (SPL Lifesciences) and were incubated for 12 h. The cells were then treated with either different concentrations of the corncob extract or vehicle for 12 h at 37 °C. Following treatment, the cells were harvested, washed in phosphate-buffered saline (PBS), fixed in 0.6 mL ice-cold 100% ethanol, and stored for 1 h at −20 °C. The cells were subsequently washed in PBS and treated with 10 mg/mL RNase A for 30 min at 37°C, followed by staining with 0.5 mg/mL propidium iodide (PI) in PBS for 10 min at 4°C in the dark. Cell cycle phase distribution was determined by using Guava EasyCyte and FlowJo Version 7.0. All experiments were repeated at least three times.

2.5. Measurement of the intracellular level of ROS

ROS was measured by using H_2DCFDA (Invitrogen) as described previously [25]. Briefly, C6 glioma cells were treated with different concentrations of the corncob extract for 24 h and were subsequently incubated with 10 μM H_2DCFDA for 30 min at 37 °C in the dark. The cells were harvested, washed with PBS three times, and were analyzed by using a Guava EasyCyte (Millipore). The mean fluorescence intensity (MFI) was determined by FlowJo Version 7.0 (TreeStar). All experiments were repeated three times.

2.6. Western blot and quantitative real-time reverse-transcription-polymerase chain reaction (RT-PCR) analysis

Western blot analyses were performed as described previously [26] with antibodies for p21, Bcl-2, MCT1, and β-actin (Santa Cruz Biotechnology). Protein expression was quantified via densitometry as previously described [27]. At least three separate experiments were conducted for each protein.

Total RNA was extracted using TRIzol Reagent (Invitrogen). Expression of mRNA was quantified by real-time RT-PCR using a StepOnePlus Real-Time PCR System (Thermo Scientific). The sequence for each primer pair are shown in Supplementary Table 1.

2.7. Statistical analysis

Data are presented as mean ± standard deviation values and were assessed by using Student's *t*-test in GraphPad Prism 5. Values were considered statistically significant at $p < 0.05$.

3. Results

3.1. Total phenol and catechin contents in corncob extract

In this study, 1 g (dry weight) of the prepared corncob extract contained approximately 94.8 mg and 1.82 μg of total phenol and

Table 1
Extraction of dried corncob and determination of total phenol and catechin content.

Extraction temperature (°C)	Extraction pressure (psi)	Total phenol (mg/g) ± SD[a]	Catechin (μg/g) ± SD[a]
110	5.69	94.77 ± 0.56	1.82 ± 0.08

[a] Data expressed as mean ± standard deviation (SD) of three replicates.