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Polysiponia morrowii Extract Inhibits Cancer Growth on CT-26 and Hela cells

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Abstract Cancer is an unfavorable human disease, and the treatment commonly have side effects and can be ineffective. Since exploration and development of cancer treatment drugs is particularly demanding, this study aimed to investigate the anticancer activities of Polysiponia morrowii extract s (PME) on CT-26 and HeLa cells. The results showed that PME inhibited cell proliferation in a dose-dependent manner, with IC50 values of 41.04% in CT-26 and 48.51% in HeLa cell cultures. Moreover, cytological observation using Hoechst 33342 staining assay showed typical apoptotic morphology in both cancer cells, and production of sub-G1 DNA was induced by PME treatment in a dose-dependent manner, with 34.41% in CT-26 and 46.01% in HeLa cell cultures. These findings suggest that PME may have potential preventive effects or medicinal value in the treatment of colorectal and cervical cancers.

Keywords : Anticancer; CT-26; Hela; ; *Polysiponia morrowii*, seaweed

Introduction

Cancer is considered to be the leading cause of death worldwide as well the single most important barrier to improve life expectancy in the 21st century [1]. In South Korea, newly diagnosed patients with cancer more than 220,000 and a quarter of deaths in 2019 were caused by cancer [2]. Colorectal cancer is the third most common malignancy in Korean, which highly increase with age for both men and women [3]. In women, cervical cancer is the fourth most common disease which denote a rising trend with age [3, 4]. Chemotherapy has been the mainstay of cancer treatment in the past 60 years, which is commonly used to treat advanced, metastatic or recurrent disease; however, current regimens suffer from intense side effects, limited response, and drug resistance [5-7]. Therefore, to explore the safer and persistently effective drugs with fewer side effects are necessary [8]. Emerging evidence indicates that lots of natural products from plants, microbes and marine organisms have beneficial effects on the prevention and treatment of cancer [9-12].

Marine organisms are important resource of marine natural products, which is a key attribute related to human health in the prevention of canc er and metabolic syndrome related obesity, diabe tes, chronic inflammation and cardiovascular dis

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ease [13-16]. Many studies have shown that the anticancer activity of algae is closely related to the content of antioxidant compounds such as polyphenols and flavonoids [17]. In Chlorophyceae, the derived extract of *Halimeda* sp. contains a lot of polyphenols, such as catechins, epicatechins, gallic acid and epigallocatechin gallate [18]. In Rhodophyceae, the alcoholic extract of *Acanthophora spicifera* showed tumoricidal activity in mouse Ehrlich ascites carcinoma cells [19]. In addition, edible seaweed extracts such as *Palmaria palmate* has been shown to inhibit the proliferation of cancer cells [20]. Furthermore, two bromophenols isolated from *Rhodomela confervoides* exhibited anticancer activities *in vitro* proliferation of selected cancer cell lines [21].

Polysiphonia morrowii Harvey (*P. morrowii*) is a species of red algae native to Northeast Asia [22]. It has become an invasive species in Europe, Australia, New Zealand, and South America [23, 24]. *P. morrowii* derived compounds have been reported some of its functions including antioxidant, anti-inflammatory, anticoagulant, antiviral and anti-microbial activities [25-29]. However, the functionality of *P. morrowii* still unclear, more importantly it is not known its anti-cancer effects yet. Therefore, in this study, we applied *P. morrowii* extract (PME) to mouse colon carcinoma cell line (CT-26) cells and human cervical cancer (Hela) cells to investigate its anticancer effects.

Materials and Methods

Chemicals and Reagents

RPMI-1640 medium, fetal bovine serum (FBS), penicillin–streptomycin, phosphate buffer saline (PBS) and trypsin–EDTA were purchased from Gibco/BRL (Burlington, Ont, Canada).
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ribonuclease A, propidium iodide (PI), dimethyl sulfoxide (DMSO), and Hoechst 33342 were purchased from Sigma (St. Louis, MO, USA).

Extract method of PME

P. morrowii was collected from Jeonbuk buangun at January 2019, rinsed with fresh water to remove the salt, epiphytes, and sand, and stored at -75℃. The frozen samples were lyophilized and finely ground. To prepare the extract, 1 g (dry weight) of the alga was solubilized in 100 mL of 80% methanol for 24 h under continuous shaking at 20℃, and then the extracts were filtered and concentrated under a vacuum in a rotary evaporator (EYELA, Tokyo, Japan) at 40℃.

Cell culture

Hela cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and CT-26 cells were purchased from the KCLB (Korean Cell Line Bank, Seoul, Korea). These cells were grown on DMEM supplemented with 10% (v/v) FBS, 10 μg/mL of adenosine, thymidine, cytidine and guanosine, penicillin (100 μg/mL) and streptomycin (100 μg/mL) at 37°C in a 5% CO_2 incubator.

Cell growth inhibitory assay

MTT assay was performed to identify whether the PME inhibit the growth of

cancer cells. Detached cells (CT-26 and Hela cells) were seeded into flat-bottomed 96 well plates at a density of 2 × 10^5 cells/mL with a final volume of 100 μl/well for 24 h at 37°C. Then, the cells were treated with PME (25, 50, 100 μg/mL) and incubated for the additional 24 h at 37°C. MTT stock solution (final 10%) was added to each well and incubated for 0.5 h at 37°C. The MTT formazan crystals were dissolved by adding dimethylsulfoxide and the optical density was measured at 570 nm by a multi-well scanning spectrophotometer (Multiskan Spectrum, Thermo Electron Co.).

Nuclear staining with Hoechst 33342

The nuclear morphologic changes of CT-26 and Hela cells was performed using the cell-permeable DNA dye Hoechst 33342 (Sigma). The cells were seeded in 24 well culture plates at a concentration of 1.0 × 10^5 cells/mL. After 24 h, the cells were treated with different concentrations of PME (25, 50, 100 μg/mL) and followed by incubation for 24 h at 37°C. The cells images were observed using a fluorescence microscope (Zeiss, city, Germany).

Flow cytometry analysis

Cell cycle analysis was conducted to determine the population of apoptotic sub-G_1 hypodiploid cells. The cancer cells were seeded at a concentration of 2 × 10^5 cells/mL in a 60-mm dish for 24 h at 37°C, and then treated with various concentrations of PME (25, 50 and 100 μg/mL) for 12 h at 37°C. The cells staining using PI solution (50 μg/mL) (Sigma, USA) and RNase A (0.2 μg/mL) (Promega, WisconsinUSA). Flow cytometry was conducted using a Beckman Coulter CytoFLEX Flow Cytometer (Beckman Coulter).

Statistical analysis

All of measurements were tested in triplicate and values are represented as the mean ± S.E. Significant differences were compared using one-way analysis, and a subsequent multiple comparison test (Tukey) was performed using Graphpad prism version 8.0 (Graphpad software, CA). Differences were considered significant at $p < 0.05$.

Results

PME induced the growth inhibitory effect against CT-26 and Hela cells

In order to determine whether the PME have anti-cancer effect, the different concentrations of PME (25, 50 and 100 μg/mL) were treated to CT-26 and Hela cells for 24 h. PME treatment inhibited the growth of both cancer cells compar ed to that of the control in a dose-dependent man ner. The 50% inhibitory concentration (IC_{50}) val ue were 41.04 μg/mL and 48.51 μg/mL on CT-2 6 and Hela cells, respectively. Our results showe d that PME compounds showed good inhibitory effect against CT-26 and Hela cells with IC_{50} val ue of 41.04 and 48.51 μg/mL, respectively (**Figu re 1**).

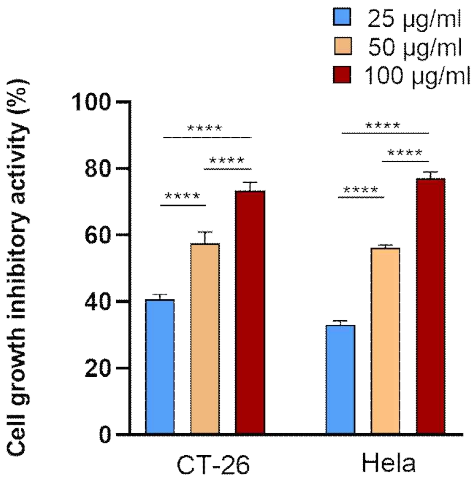

Figure 1. Inhibitory effects of PME on growth of CT-26 and Hela cells. The cells (2×10^5 cells/mL) cultured with PME (25, 50, 100 μg/mL) for 24 h and MTT assay was performed. The results are representative of three separate experiments (n = 3). **** $P < 0.0001$.

PME induced the apoptotic body formation and sub-G_1 DNA content in CT-26 and Hela cells

Next, we examined whether PME treatment in duces cell death in CT-26 and Hela cells using Hoechst 33343 staining. The morphological obse rvation in the cell nuclei of CT-26 and Hela cells showed morphological alterations including segr egated and apoptotic bodies formation in a dose-dependent manner **(Figure 2A, 3A)**. And, we exa mined sub-G1 DNA content by treatment of PM E using flow cytometry. PME-treated both CT-2 6 and HeLa cells increased sub-G1 populations (apoptotic cells) compared with that of the contr ol, and the proportions of sub-G1 phase were recorded to 34.41% and 46.01%, respectively in 100 μg/mL PME treatment cells **(Figures 2B, 3 B)**.

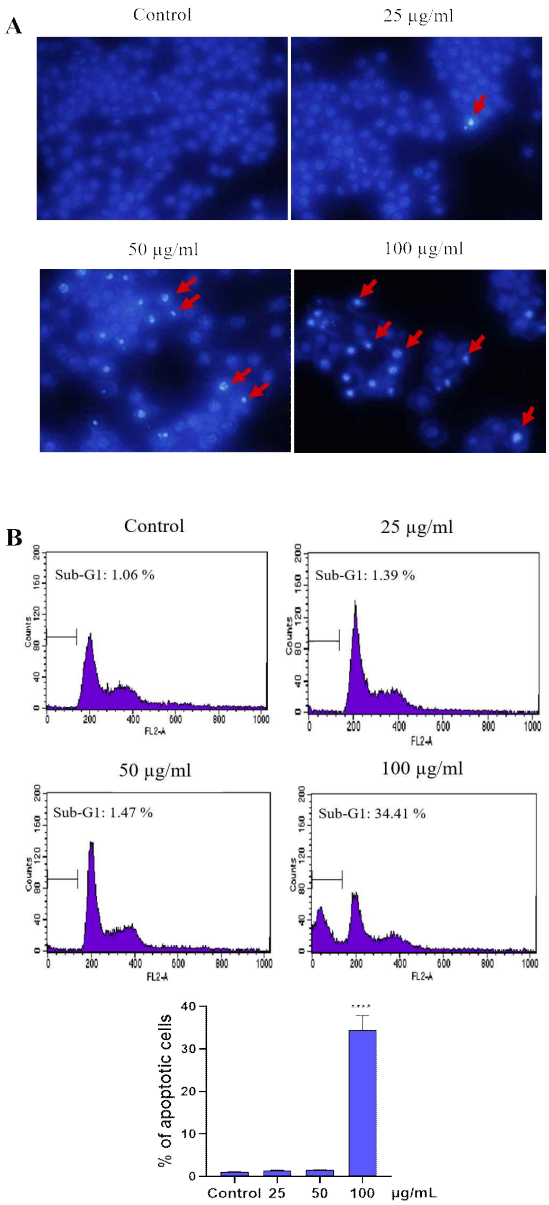

Figure 2. PME-induced apoptosis in CT-26 cells. The cells were incubated with PME (25, 50, 100 μg/mL) for 24 h. (A) Microscopic images of cell apoptosis were measured by Hoechst 33342. The apoptotic cell s are characterized by brighter staining of chromatin condensation and fragments (arrows indicate apoptoti c cells). (B) The proportion of sub-G1 DNA producti on in CT-26 cells were analyzed by flow cytometry and the bar graph presenting the percentage of apopto tic cells. Data were presented as the mean ± SD. **** $P < 0.0001$.

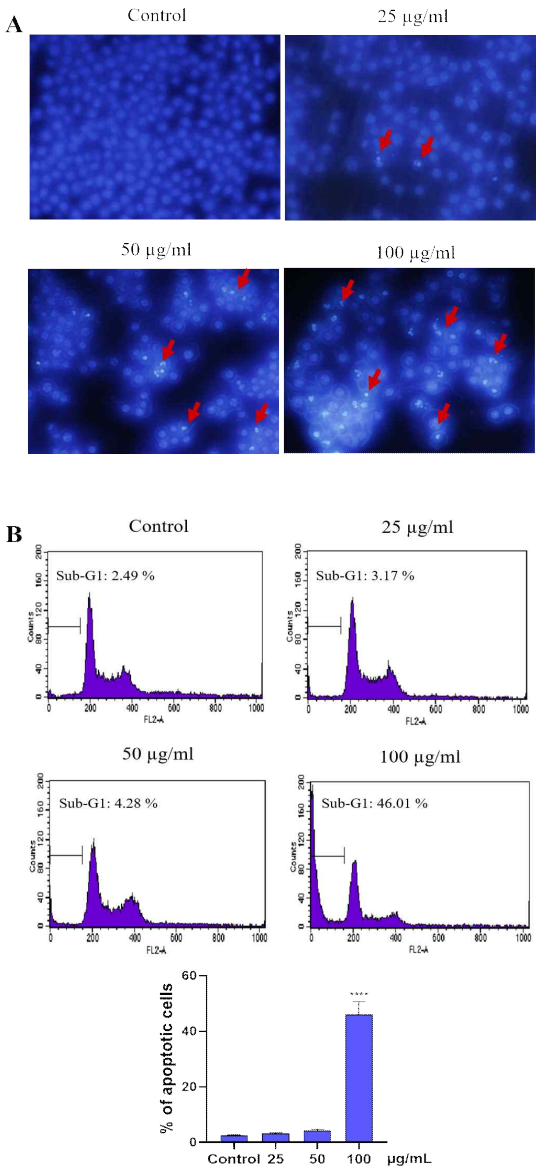

Figure 3. PME-induced apoptosis in Hela cells. The cells were incubated with PME (25, 50, 100 μg/mL) for 24 h. (A) Microscopic images of cell apoptosis were measured by Hoechst 33342. The apoptotic cell s are characterized by brighter staining of chromatin condensation and fragments (arrows indicate apoptoti c cells). (B) The proportion of sub-G_1 DNA producti on in Hela cells were analyzed by flow cytometry and the bar graph presenting the percentage of apopto tic cells. Data were presented as the mean ± SD. **** $P < 0.0001$.

Discussion

So far, marine natural products have been recognized as the most valuable candidate of pharmaceutical materials [30, 31]. Seaweeds presented significant antiproliferative activity on various tumor cell lines [32-34]. However, no studies have been concerned to evaluate the anticancer activities of fractions or compounds isolated from *P. morrowii*. Previous study found that *P. morrowii* derived the 80% methanolic extract (3-bromo-4,5-dihydroxybenzaldehyde, BDB) exhibited strong anti-IPNV and anti-IHNV activities [35]. The compound of BDB isolated from marine red alga, *Rhodomela confervoides*, were found to have selective antitumor activities against KB, Bel 7402 and A549 tumor cells [21]. Another study isolated a novel bromophenol [Bis (3-bromo-4,5-dihydroxybenzyl) ether, BBDE] from *P. morrowii* which inhibited inflammation in RAW 264.7 macrophage cells [36].

In the present study, PME derived from a red algae of *P. morrowii*, was found to have inhibitory effects on the growth of CT-26 and Hela tumor cells, and their IC_{50} values of 41.04% (CT-26 cells) and 48.51% (Hela cells) also showed the anticancer properties. Antioxidant compounds such as polyphenols and flavonoids in seaweed are implicated with their anticancer activities [18]. Flavonoids can prevent cancer by affecting the signal transduction pathway of cell proliferation, antioxidant activity, regulating the enzyme activity related to estrogen biosynthesis and the metabolic pathway of carcinogens [37]. Polyphenolic compounds suppressed cancer cells by altering the metabolism of potential carci-

nogens through xenobiotic metabolic enzymes [17]. Another evidences indicated that polyphenolic compounds change the metabolism of many potential carcinogens by activating a variety of xenobiotic enzymes, interfering the telophase stage of mitosis, reducing the mitotic index as well as colony forming unit of tumor cells [38, 39]. However, further studies are necessary to clarify the anticancer component(s) of PME in *P. Morrowii* and its mechanisms of inhibiting the growth of CT-26 and Hela cancer cells.

Cell proliferation and apoptosis are two factors that deciding the growth of cancer cells [40]. We speculated that the decrease of cell viability after treatment may be due to the PME-induced cell death or attribute to the inhibition of biochemical or biological functions of cells exposed to PME. In this study, cells treated with PME presented typical features of early apoptosis which could observed as crescents around the nucleus, or the entire chromatin was appear as one or a group of or featureless bright blue spherical beads [41]. Furthermore, the proportion of sub-G_1 phase (apoptotic cells) were significantly increased by treatment with PME at higher concentrations, our results demonstrated that PME treatment inhibits the growth of cancer cells through increasing the formation of apoptotic body and sub-G_1 DNA population, eventually lead to subsequent apoptosis in CT-26 and Hela cells (**Figure 2, 3**). Our study results are similar to previous anticancer studies [42] that have demonstrated the effect of colon cancer cells growth suppression by inhibiting nuclear transcription factors. Therefore, this seaweed has potential as an anticancer effect, and is expected to be an important marine resource for anticancer function research in the future.

Conclusions

PME could remarkably inhibit the proliferation of CT-26 and Hela cancer cell lines in a dose-dependent manner, which also induce the apoptosis and increase their sub-G_1 populations. These results suggest that PME has anticancer activity and presents a promising prospect for research and development. However, the development of PME into a clinically effective anti-cancer drug requires further studies.

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