Anticancery Activity In Vitro Polysaccharides Fermented from Brown Algae

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Abstract. Cancer has become one of the world's leading causes of death, and the current treatment of cancer still cannot evade the side effects of chemotherapy drugs. In this study anticancer effect of fucoidan isolated from brown seaweeds polysaccharide was investigated. The obtained seaweeds polysaccharide is mainly prepared by fermenting, separating and purifying seaweeds by a mixed strain of yeast and Lactobacillus plantarum. Polysaccharides in algae can inhibit or kill tumor cells by enhancing the body's immune function and defense capability. Intake of polysaccharides can activate or activate macrophages, thus inducing them to produce tumor necrosis factor. Polysaccharides extracted from seaweed were formulated into different concentrations of drugs to treat 231 cells and HCT-116 cells to detect the effects of extracts on the two cancer cells. When no drug is added to the cell suspension, the cell grows well. The polysaccharides had no cytotoxicity on HCT-116 cells under 500 µg/mL and inhibited cell viability at concentration of 2000 µg/mL. As for 231 cells, the cell survival rate was 61.08% at 1000 µg/mL. The present research clearly indicated the cytotoxicity of seaweeds polysaccharide in HCT-116 cells and 231 cells through adding them. These results also suggested the seaweeds polysaccharide may be a powerful agent against cancer cells.

1. Introduction
Cancer has become one of the most fatal diseases in the world, and the prospect of its occurrence is not optimistic. According to "Cancer Facts & Figures2015", the number of new cancer cases worldwide will reach 21.7 million and the number of deaths will reach about 13 million by mid-2030 [1]. Cancer incidence in China is also very severe. Among all cancer types, colon cancer and breast cancer are one of the ten most common tumors in the whole body [2]. At present, there is no definite and effective therapeutic drug for cancer, so it is vital important to develop the safe and effective anti-cancer drugs and technologies [3].

Colon cancer is a common malignant tumor of digestive tract occurring in colon, and its incidence rate is the third highest among gastrointestinal tumors [4]. Colon cancer is mainly adenocarcinoma, mucinous adenocarcinoma and undifferentiated carcinoma. Chronic colitis patients, colon polyps’ patients and obese men are susceptible groups [5].
Breast cancer is a malignant tumor that occurs in the epithelial tissue of the breast gland [6]. In situ breast cancer is not fatal. However, when breast cancer cells lose the characteristics of normal cells, the connections are loose and easily detached. Once the cancer cells fall off, the free cancer cells will spread through the blood or lymph to the whole body, forming a metastasis and endangering life [7]. In recent years, the growth rate of breast cancer in China has increased by 1-2 percentage points compared with high-risk countries.

Probiotics and algae derivatives (i.e., fucoidan, seaweed fiber, and brown algae starch) play important roles in many biological processes, such as cell proliferation, differentiation and apoptosis, and immune responses [8]. Seaweed supernatants also function in cells, and their bioavailability has been shown to inhibit the rate of proliferation of colon and breast cancer cells [9]. In addition, probiotics are active microorganisms that promote human health. It regulates and improves the intestinal flora when the body eats enough food. A large body of literature has proven that probiotics not only regulate the structure of the intestinal flora, but also have anti-bacterial and anti-inflammatory effects [10]. One of the most studied polysaccharides in kelp is seaweed polysaccharides with complex composition and various biological activities [11]. It is a kind of multi-component mixture with extensive anti-bacterial [12], anti-parasitic [13], anti-cancer [14], anti-oxidant [15], anti-inflammatory and other properties [16,17]. Its biological activity also includes the regulation of immune function [18]. Today, *seaweeds polysaccharide* is widely used as a clinical treatment for various diseases because of its low side effect and drug resistance. Therefore, the present study focused on investigating the potential anticancer activity of *seaweeds polysaccharide*.

2. Materials and methods

2.1. Preparation of Algae Polysaccharide

Accurately weigh 1000g of kelp, wash and pulverize into small pieces, add 20L of water according to the ratio of 1: 20, add potassium dihydrogen phosphate, acid cellulase and acid pectinase according to the ratio of raw materials, measure its pH, adjust it to 4.8-5.0, stir at 50 ℃ for 2h, add sodium carbonate and alkaline pectinase, stir at 60 ℃ for 1.5h, finally add water to 30L, make the total system 30 times of the raw materials, continue stirring for 50min, centrifuge at 3700r/min to obtain supernatant, and preserve it at 4 ℃ for later use.

After sterilization, the inoculated plant *Lactobacillus* and *Yeast* mixed bacteria were inoculated in 2%, 37 ℃ 300r/min, and the aeration ratio was 25% continuous fermentation for 2 days sampling, lyophilized, and powdered to obtain seaweed supernatant powder. The dried extract was used directly for the study.

2.2. Cell culture

Human colorectal HCT-116 cancer cells and 231 cells were purchased from the Qilu University of Technology Molecular and Cell Culture Room. The cells were routinely maintained in RPMI-1640 medium supplemented with 10% (v/v) FBS, and 1% penicillin-streptomycin in a humidified CO2 incubator (model 3154; Forma Scientific, Inc., Marietta, OH, USA) with a 5% CO2 atmosphere at 37 ℃ and the solution was changed every other day. When the cell coverage area in the culture flask reaches 80% ~ 90%, 0.25% trypsin is added and subcultured at a ratio of 1: 2. The 4th generation cells were randomly divided into blank group, control group and seaweed polysaccharide group with different concentrations.

2.3. Cell viability assay (MTT)

Cell viability was measured using an MTT assay. HCT-116 cells and 231 cells were seeded in 96-well plates (Shandong Lemon Biological Co., Ltd.) at a density of 1×10⁴ cells/well. Following a 24 h incubation, the cells were treated with the various concentrations (250, 500,1000 and 2000 µg/ml) of *seaweeds polysaccharide* for 24 h. Following incubation with *seaweeds polysaccharide* 1mg/ml of MTT reagent (100 µl) was added to each well and the cells were incubated in a humidified incubator at
37°C to allow the MTT to be metabolized. At total of 4 h later, formazan crystals were dissolved with dimethyl sulfoxide (100 µl in each well). Absorbance of the samples was measured at a wavelength of 570 nm using a microplate reader (model, 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

3. Results

3.1. seaweeds polysaccharide cytotoxic analysis in HCT-116 and 231 cells.

The experimental results of show that the cell survival rate decreases gradually with the increase of seaweed extract concentration. When the concentration reaches 2 mg/ml, the cell death rate is close to half. It can be seen that the cell death rate increases with the increase of sample concentration (p < 0.05, Fig.1 and Fig.2). This shows that seaweed extract has inhibitory effect on HCT-116, and the higher the concentration of the sample, the stronger the inhibitory effect will be.

![Figure 1](image1.png)

**Fig.1.** Effect of seaweeds polysaccharide on OD570 value of HCT-116 cells. Date presented as mean ± SE (n=6). There is a significant difference in the bar chart with an asterisk (p < 0.05).

![Figure 2](image2.png)

**Fig.2.** Effect of seaweeds polysaccharide on OD570 value of 231 cells. Date presented as mean ± SE (n=6). There is a significant difference in the bar chart with an asterisk (p < 0.05).
4. Conclusion and Discussion
This investigation provides evaluation effectof in vitro cytotoxicity properties of a seaweed’s polysaccharide isolated from brown seaweed. The isolated seaweeds polysaccharide exhibited potent anti-cancer activity against human breast cancer cell line and colon cancer cell line. Seaweeds polysaccharide showed anti-proliferative properties at 250ug/mL, the algae supernatant inhibited the two cancer cells compared with the normal group, and the inhibitory effect on the activity of the tumor cells was more obvious with the increase of the concentration of the seaweed supernatant. In the 2 mg/mL dose group, the inhibition of the two cells reached a maximum, and the inhibition rates were 55.39% and 53.48%, respectively. Hence, the present findings suggested that the seaweeds polysaccharide isolated from Seaweeds does possess chemo preventive ability against breast cancer and colon cancer.

At present, there are many methods to detect cell activity, such as isotope method, doping method, MTT method, etc [19]. However, isotope method is radioactive [20], doping method has many steps and requires special equipment [21], etc. Among them, MTT method has the characteristics of high sensitivity, good stability, simplicity and rapidity [22], etc. It is commonly used in the screening of anti-tumor drugs and the determination of cytotoxicity experiments. MTT method is the most commonly used method to measure cell proliferation activity at home and abroad. Cell proliferation activity is an important indicator to reflect the strength of cell vitality [23]. When cells are damaged by some abnormal factors or inhibited by drugs, the first manifestation is the reduction of proliferation activity. The number of living cells is determined by measuring its A value at 570nm wavelength with an enzyme reader. The larger the OD570 value is, the stronger the cell activity is [24].

This experiment is a preliminary test on the anti-tumor activity of seaweed extract. The results show that it has a certain inhibitory effect on the growth of cancer cells. The main task of further research is to study the mechanism of the inhibition of seaweed extract on cancer cells.

Acknowledgements
The work was financially supported by Shandong Provincial Key Research and Development Program (No. 2017YYSP029, 2017YYSP010, 2018YYSP022, 2018YFJH0404).

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