

***Dunaliella salina* Exhibits an Antileukemic Immunity in a Mouse Model of WEHI-3 Leukemia Cells**

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ABSTRACT: *Dunaliella salina* has been shown to have antioxidant property and induce apoptotic cell death of human cancer cells in vitro. However, there is no information available on *D. salina* showing an antileukemia effect or immunomodulatory activity in vivo. This study applied *D. salina* to syngeneic leukemia-implanted mice (BALB/c and WEHI-3) to investigate its immunological and antileukemia properties. Oral administration of *D. salina* (184.5, 369, and 922.5 mg/kg) inhibited spleen metastasis and prolonged the survival in BALB/c mice that had received an intravenous injection of WEHI-3 cells. The results revealed that *D. salina* had reduced spleen enlargement in murine leukemia. It had also increased the population and proliferation of T-cells (CD3) and B-cells (CD19) following Con A/LPS treatment on flow cytometry and MTT assay, respectively. Furthermore, *D. salina* increased the phagocytosis of macrophages and enhanced the cytotoxicity of natural killer cells on flow cytometry and LDH assay. Moreover, *D. salina* enhanced the levels of interferon- γ and interleukin 2 (IL-2) but reduced the levels of IL-4 and IL-10 in leukemic mice. In conclusion, these results demonstrated that the application of *D. salina* had beneficial effects on WEHI-3 leukemic mice by prolonging survival via modulating the immune responses.

KEYWORDS: *Dunaliella salina*, antileukemia, immune responses

■ INTRODUCTION

Algae have been widely consumed for a long time as nutritional or pharmaceutical agents because of their biofunctional substance. *Dunaliella salina* is a halophilic unicellular microalga from the Chlorophyceae class. Significant amounts of three valuable products, glycerol, β -carotene, and proteins, are accumulated when the alga experiences high salinity or high intensity of light stress. As *D. salina* lacks a cell wall, it is easily digested.^{1–3} According to mutigenerational and toxicological studies, *D. salina* is safe for human consumption.^{3,4} As *D. salina* contains abundant β -carotene, it has been utilized as a functional food (i.e., pro-vitamin A supplement and hepatoprotective food).^{1,5–7} β -Carotene has been investigated for its anticancer properties, both in vitro and in vivo.^{8–12} As *cis* bonds are more reactive than *trans* bonds, the 9-*cis* isomer has been shown to be more antioxidative. In contrast, *all-trans*- β -carotene and α -carotene are more easily absorbed than the 9-*cis* β -isomer.^{13,14} Our previous analysis showed that the major carotenoids in *D. salina* were *all-trans*- β -carotene and 9- or 9'-*cis*- β -carotene.^{5,15} When the extract of *D. salina* was compared with pure *all-trans*- β -carotene, α -carotene, lutein, and zeaxanthin, the extract of *D. salina* showed the highest antioxidant activity.⁵ We also showed that *D. salina* suppressed lipopolysaccharide-stimulated inflammation in vitro¹⁵ and ameliorated carbon tetrachloride or UV-B-induced oxidative stress in vivo.^{6,16}

Immune responses to tumors include humoral and cellular immunity.^{17–19} B-cell-mediated humoral immune defense is a specific antigen antibody reaction. The reaction can neutralize toxins and protect against pathogen-induced infection. T-cells also release many regulatory factors including lymphotoxin,

macrophage mobile factor, interferon, transfer factor, etc. These factors can promote differentiation and proliferation of immune cells, activity of natural killer cells (NK cells), and phagocytosis of macrophages. Thus, both antibody- and cell-mediated immune defense contribute to anticancer potential.¹⁹

Recently, the anticancer activity of *D. salina* had been investigated in various cancer cells including lung,⁹ skin, and prostate cancer cell lines.⁸ We hypothesized that *D. salina* might induce apoptosis, differentiation, and immunomodulation activities against leukemia. As there had been no report on the effects of *D. salina* on immunomodulatory responses or on antileukemia activity in vivo, our study focused on these aspects of *D. salina*. The syngeneic leukemia-implanted mice (BALB/c versus WEHI-3) had been used for evaluating the antileukemia activity of drugs and natural products for many years. In this study, *D. salina* has been shown to have antileukemia properties via increasing the immunity in WEHI-3 leukemic mice.

■ MATERIALS AND METHODS

Materials and Reagents. Commercially available spray-dried preparations of *D. salina* were cultured under light and 20% salt cultivation in the outdoor cultivation pool at GONG BIH Enterprise Co., Ltd. (Yunlin City, Taiwan, ROC). The contents of water, protein, fat, ash, sugar, and dietary fiber were 40, 466, 220, 57, 130, and 87 mg/g algae, respectively. The quality and authenticity of *D. salina* powder were described and provided by the company. The carotenoid

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