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Journal of Cancer Immunology Commentary

Prevention of Lung Cancer Growth by Water Extract from *Euglena gracilis*

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Commentary

As a source of novel drugs and dietary supplements, natural products from plants and animals have been studied for more than decades. Unicellular algae, such as *Chlorella*, *Spirulina* and *Euglena*, are such resources. *Euglena gracilis* is a unicellular green alga found in fresh water. This organism possesses features characteristic of both animals and plants, having the ability to swim by means of flagella and to photosynthesize. This alga contains a rich variety of nutrients including amino acids, carbohydrates, vitamins, and minerals; therefore, it has been used as a nutritional and functional dietary supplement [1]. Furthermore, it has been shown that a dried powder of *Euglena* species or its extracts in water or polar organic solvents have potential medicinal properties, such as antimicrobial [2], anti-mutagenic , anti-inflammatory [3,4], anti-fibrotic [5], anti-viral [6], anti-obesity [3,7], and antitumor [8-10] activities. It has been hypothesized that carbohydrate granules made by *Euglena* for energy storage and consisting mainly of β-1,3-glucan, called paramylon, are primarily responsible for these biological activities. However, our recent study demonstrated that a partially purified water extract from *Euglena gracilis* devoid of mature paramylon granules (referred to hereafter as *Euglena* water extract or EWE) inhibited the growth of lung cancer cells in culture and in a mouse orthotopic lung carcinoma allograft model [11]. In cell culture, the direct attenuating effect of EWE on lung cancer cell lines was observed in 2-dimentional (2D) and 3-dimentional (3D) spheroid culture. In 3D spheroid culture, spheroid growth of the lung cancer cells was significantly attenuated by EWE treatment compared with the PBS control group. However, EWE did not stop the spheroid growth completely suggesting that the direct cancer cell growth inhibition by EWE may be effective only in the early stage of tumor growth. In a mouse orthotopic lung carcinoma allograft model, orally administered EWE, which was started 3 weeks prior to cancer cell inoculation, prevented growth of lung carcinoma by attenuating immune regulatory cell populations including granulocytes and myeloid-derived suppressor cells (MDSCs). This preventive effect may function not only in early stage of tumorigenesis, but may last into later stages of tumor development *in vivo*. The notable findings in this study are (1) orally administered EWE that does not contain mature paramylon granules exhibited a preventive effect against lung tumor growth in mice, (2) EWE attenuated populations of myeloid-derived suppressor cells in cell culture and granulocyte populations in peripheral blood of mice. Both of these cell types (myeloid-derived suppressor cells and granulocytes) play an important role in immune suppression; hence, reduction in their populations may be linked to enhanced immune activity against cancer. Together, these results suggest that *Euglena* water extract contains a cancer growth prevention agent. However, many questions remain about the nature of this agent including: (1) Is this anti-cancer activity due to a single component of *Euglena gracilis* or might it be a synergistic effect of multiple components? (2) What is the chemical nature of this agent or agents? (3) Is there involvement of β-1,3-glucan (the major component of paramylon) in this unique bioactivity? (4) How does this agent attenuate granulocyte genesis? (5) Is intestinal microbiota involved in induction of this bioactivity? and (6) What is the overall effect of this agent on animal health? In this commentary, we discuss evidence suggesting that paramylon (and the β-1,3-glucan of which it consists) may not be primarily responsible for EWE bioactivity, evidence indicating the involvement of intestinal microbiota, and the overall effect of EWE on animal health. The exact chemical nature of the bioactive agent and potential mechanisms by which EWE attenuates granulocyte genesis will be addressed in future work.

The lack of involvement of β-1,3-glucan and paramylon in lung cancer prevention by EWE

Paramylon consists of water-insoluble β-1,3-glucans having molecular masses on the order of 500 kDa. The size of paramylon granules ranges from 1-6 μm in diameter and they typically constitute approximately 80% of *Euglena* body weight [1,11]. Due to their large size and solid granular shapes, paramylon can be purified easily by low speed centrifugation from a suspension of whole *Euglena* dry powder in water [11]. It has been suggested that paramylon acts as an immunostimulant or an immunopotentiator [12-14]. In previous studies, paramylon was shown to stimulate production of pro-inflammatory cytokines, such as IL-1, IL-6, tumor necrosis factor (TNF), etc. [12-14]. In our study, we demonstrated that *Euglena* water extract retained its cancer-prevention activity even after removal of a majority (if not all) of the paramylon granules by multistep centrifugation and filtration through a 0.22 μm pore size filter [11]. However, it is impossible to rule out a potential contamination with the immature paramylon granules with less than 0.2 μm diameter and water soluble β-1,3-glucan oligomers in the EWE. Gissibl et al. reported that paramylon-associated bioactivity was maximized when the paramylon granules were hydrolyzed by heat, enzymes, or acid treatment into water-soluble short chain β-1,3-glucan oligomers [15]. Indeed, they detected increased bioavailability of hydrolyzed paramylon and better protection against infection by *Staphylococcus aureus* or *Candida albicans via* increased serum IL-12 production, resulting in longer survival of mice. However, since a heated water extract prepared from purified paramylon granules did not show any lung cancer growth prevention in a parallel mouse study (our unpublished data), the EWE-dependent prevention of lung cancer growth that we observed is not likely attributable to contaminated paramylon nor water-soluble β-1,3-glucan oligomers.

Involvement of intestinal microbiota in lung cancer prevention by EWE

Although the lung cancer preventive agent in the EWE has yet to be identified, involvement of intestinal microbiota or their metabolites in its cancer prevention is postulated. Because EWE was administered through drinking water in our study, it is conceivable that the EWE altered both the quantity and quality of the intestinal microbiota as well as its metabolites. The intestinal microbiota of humans, typically consisting of about 10^{14} living organisms [16,17], has been shown to generate large amounts of various metabolic products including substances beneficial for host health (vitamins, short-chain fatty acids (SCFAs), etc.), as well as other substances in potentially harmful quantities (amines, hydrogen sulfide) [18]. It has been shown that the intestinal microbiota and its metabolites have an important role in maintaining host homeostasis and health [18,19]. Among these intestinal microbiota metabolites, SCFAs can act as ligands for G protein-coupled receptors (GPCRs) [20]. Because GPCRs are involved in many essential biological reactions, GPCRs are often targets for therapeutic drugs [21,22]. Furthermore, SCFAs are known to influence the development and function of the immune system [23]. SCFAs reduce expression of T cell-activating molecules in innate immune cells such as macrophages and dendritic cells by inducing histone deacetylase (HDAC) inhibition. This HDAC inhibition by SCFAs also influences peripheral T cells, particularly regulatory T (Treg) cells. In a mouse study, HDAC inhibition by SCFAs increased forkhead box P3 (FOXP3) expression in Treg cells and enhanced the suppressive function of $FOXP3^+$ Treg cells, following amplified Treg cell-mediated attenuation of colitis [24]. These mechanisms are crucial for maintaining immune homeostasis. In addition, Harusato et al. demonstrated that exposure to beneficial microbiota in early-life was important in establishing intestinal homeostasis that restrains colon cancer in adulthood [25]. This regulation against colon cancer appears to be associated with an alteration of granulocytic MDSC in colonic lamina propria. Another investigation demonstrated that an intestinal microbiota component species, *Fusobacterium nucleatum*, contributed to promotion of growth of colorectal cancer by increasing MDSC in the tumor site [26]. Since EWE-induced lung cancer growth prevention required preliminary administration of the EWE three weeks prior to allografting, no lung cancer growth prevention was observed when the EWE was administered after allografting. We suspect that the effect of EWE is primarily by alteration of immune system homeostasis. Due to accumulated experimental evidence that oral administration of micro-algae alters the intestinal microbiota as well as its metabolites [27,28], EWE-dependent lung cancer growth prevention is potentially mediated by these alterations in intestinal microbiota and excreted metabolites.

Neutrophils in lung cancer prevention by EWE

Oral administration of EWE significantly attenuated the granulocyte population in mouse peripheral blood. In addition, this administration also directly attenuated granulocytic MDSCs in a primary cell culture with mouse bone marrow cells [11]. Neutrophils, which account for a majority of the granulocytes, play a fundamental role in the innate immune response. Neutrophils immediately migrate to the inflammation site and kill invading bacterial and fungal species through phagocytosis, by release of preformed granular enzymes and proteins, and by the production of a range of reactive oxygen species [29]. It is also known that tumor-associated neutrophils (TANs) play important roles in the tumor microenvironment, having both pro- and anti-tumor effects [30]. Transforming

growth factor-β (TGF-β), an immunosuppressive cytokine released from tumors, transforms tumor-infiltrating neutrophils to an N2 pro-tumorigenic phenotype, while they are transformed back into an N1 anti-tumorigenic phenotype by a TGF-β blockade. The N2 TANs attenuate T cell activity, therefore, promoting tumor growth [30]. A high neutrophil-lymphocyte ratio in both peripheral blood [31-34] and in the tumors [35-37] is highly correlated with poor prognosis in chemo- and immunotherapy in patients with malignant tumors. Therefore, the decrease of the granulocyte population in mouse peripheral blood observed in our study may suggest that EWE intake generates a favorable condition for antitumor immunity in the host. No leukocyte analysis in the tumor tissues was carried out in this study. Future work to compare the immune cell populations in both peripheral blood and tumor tissues in mice with or without EWE treatment may provide a better understanding of the antitumor effect of EWE. On the other hand, a large decrease in the neutrophil population of peripheral blood, called neutropenia, causes severe health problems in both human and animals [38,39]. Neutropenia patients have an increased risk of infection by bacterial and fungal species. In cancer, treatment with radiation therapy, chemotherapy, or immunotherapy sometimes cause severe infectious disease due to neutropenia [40,41]. No noticeable health problems were observed over five to six weeks of EWE administration in our mouse studies. The effect of EWE administration on the granulocyte genesis should be studied carefully in the future using other animal species since the neutrophil population in mouse peripheral blood constitutes only 15% of the whole leukocyte population ([42], average of 42 inbred strains of mice), which is significantly smaller than the 70% fraction in human peripheral blood [43].

Conclusion

The water extract from *Euglena gracilis* dry powder devoid of mature paramylon granules, abbreviated EWE, prevented the growth of lung carcinoma in mice. Oral administration of the EWE may have altered intestinal microbiota and metabolites and attenuated granulocytic MDSC and granulocyte in peripheral blood, thereby preventing lung cancer growth in mice. Although its chemical nature is yet to be determined, a water soluble β-1,3-glucan or paramylon is not likely to be the cause of this bioactivity. It is urgently important to identify this bioactive substance and study both the safety and utility of EWE-dependent cancer growth prevention.

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