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Polysaccharides from *Dioscorea* (山藥 Shān Yào) and Other Phytochemicals Enhance Antitumor Effects Induced by DNA Vaccine Against Melanoma

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ABSTRACT

Adjuvants can be used to enhance the immunogenicity of antigens and improve the efficacy of vaccines. Potent adjuvant action is known to often correlate with the activation of the transcription factor, nuclear factor-κB (NF-κB). Specific plant polysaccharides and a variety of phytochemicals from foods and traditional medicinal herbs have been shown to modulate NF-κB activation. In the present study, selected plant polysaccharides and phytochemicals were evaluated for use as a DNA vaccine adjuvant in a murine melanoma model. We observed that a specific ethanol extract fraction (DsCE-I) from the tuber of a key Traditional Chinese Medicine plant, *Dioscorea* (山藥 Shān Yào), enhanced the protection against melanoma after immunization with a gene-based vaccine. A number of anti-inflammatory phytochemicals tested were able to partially diminish the inflammation-associated tumorigenesis elicited by LPS. Among the several phytochemical combinations investigated, the use of an adjuvant containing LPS in combination with emodin resulted in smaller tumors and higher survival rate in test mice than the use of other adjuvant treatments and the control sets in this DNA cancer vaccine model. A *Dioscorea* polysaccharide fraction (DsCE-I) and several specific phytochemicals warrant further exploration as useful adjuvants for anticancer vaccines.

Key words: Adjuvants, Anticancer vaccine, *Dioscorea*, Phytochemicals

INTRODUCTION

Traditional Chinese medicines (TCM), or as part of so-called "complementary and alternative medicines" (CAM) by Western medical communities, are being increasingly recognized and used in public health care throughout the world. Government- and industry-funded research and development have started to focus on the use of scientific evidence-based approaches to verify the traditionally claimed medicinal efficacies, specific bioactivities, and related pharmacological mechanisms of such remedies. In

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Asia, *Dioscorea* spp. (山藥 Shān Yào) are very popularly used as a health food/supplement and/or as a TCM herb that can be taken alone or in multiple-herb formulations. They are used for a broad range of ailments or health care measures. Some specific biological effects have been reported for *Dioscorea* spp., including antitumor,[1] induction of hypoglycemia in experimental mice and rabbits,[2,3] antibacterial,[4] as well as antioxidative and hypolipidemic activities.[5] Anecdotal evidences suggest that *Dioscorea* tubers taken as a food supplement may promote human health by regulating and upgrading the immune responses[6] and promoting antitumor activities;[1] however, credible experimental results and related mechanisms are still very limited.

Suppression of tumor progression by functional bioactivities of secondary metabolites from plants has been shown to confer anticancer or chemoprevention activities. Previously, we reported that co-treatment with a 50-75% ethanol-partitioned fraction of the tuber crude extract of *Dioscorea batatas* (DsCE-II) and interleukin-2 (IL-2) resulted in a significantly higher rate of murine splenocyte cell proliferation *ex vivo* than treatment with DsCE-II or IL-2 alone. This DsCE-II fraction, which contains a polysaccharide with a high proportion of β-1,4 linkage mannose (≥64%), also promoted the regeneration of specific progenitor cell populations in damaged bone marrow tissues of 5-fluorouracil–treated mice.[7] In addition, DsCE-I, a 50% ethanol-insoluble fraction of *D. batatas*, significantly increased granulocyte-macrophage colony stimulating factor (GM-CSF) promoter activity in normal and inflamed skin,[8] suggesting that DsCE-I may be useful as an adjuvant for use alongside chemotherapy in cancer.

Potent adjuvant action often correlates with nuclear factor-κB (NF-κB) activation, as exemplified by monophosphoryl lipid A (MPL).[9] However, NF-κB activation is also known to play an important role in the development and/or maintenance of various types of cancer. NF-κB has been linked to cell proliferation, invasion, angiogenesis, metastasis, suppression of apoptosis, and chemoresistance in multiple tumor systems.[10,11] Most carcinogens, inflammatory agents, and tumor promoters, including tar components from cigarette smoking, phorbol ester, okadaic acid, H_2O_2, and tumor necrosis factor-alpha (TNF-α), have all been shown to activate NF-κB. In addition, NF-κB has been shown to regulate the expression of a number of genes whose products are involved in tumorigenesis. Therefore, the balance of NF-κB in anticancer immune responses and tumorigenesis is a critical and tricky issue in cancer therapy.

The present study investigated the adjuvant effect of DsCE-I in protecting against B16-hgp100 melanoma in a DNA vaccine model. In addition, several anti-inflammatory phytochemicals known for their anti–NF-κB activity were investigated for their ability to diminish the inflammation-associated tumorigenesis in this vaccine model study.

MATERIALS AND METHODS

Preparation of *Dioscorea* plant tuber crude extract (DsCE-I) using ethanol partition

Tuber tissues of the *Dioscorea* plant, *D. batatas* Decne (山藥 Shān Yào), were used to prepare the ethanol extract DsCE-I. The authenticity of all plant materials and species verification was validated by Dr. Sin-Yie Liu, Taiwan Agricultural Research Institute. Cultivation, growth, taxonomy, and agricultural practice details have been previously reported.[12] The preparation of DsCE-I was conducted as described previously.[8] In brief, 10 g tuber powder was mixed with 100 ml Milli-Q water, stirred for 1 h at room temperature, and centrifuged at 24,000 g for 20 min at 4°C. The supernatant was filtered through glass wool. The pellet was resuspended with another 100 ml water, stirred, centrifuged, and re-extracted as above. The supernatants from two extractions were then pooled to yield a crude extract (CE) fraction. The CE fraction was further extracted stepwise with 50% (V/V) ethanol. The ethanol-insoluble fractions were collected by centrifugation at 24,000 g for 20 min at 4°C; the pellet was lyophilized and then dissolved in sterilized water at 10 mg/ml. The fractions were named DsCE-I. *Limulus amoebocyte* lysate (LAL) assays (associates of Cape Cod, Falmouth, MA, USA) were performed to detect possible endotoxin contamination.

Reagents

Pyrrolidine dithiocarbamate (PDTC), SB203580, Lipopolysaccharide (LPS, *Escherichia coli* 055:B5), polymyxin B, forskolin, friedelin, oleanolic acid, resveratrol, and nidosamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Emodin was purchased from ACROS Organics (Fair Lawn, NJ, USA). Liquiritigenin was purchased from Extrasynthese (Lyon, France). Honokiol was purchased from Pharmaceutical Industry Technology Development Center (New Taipei City, Taiwan).

Mice

Female C57BL/6JNarl mice (6-8 weeks old), which were purchased from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan, were maintained under standard pathogen-free conditions. All facilities were approved by the Academia Sinica Institutional Animal Care and Utilization Committee (IACUC), and all animal experiments were conducted under the institutional guidelines established by the Animal Core Facility and IACUC in Academia Sinica, Taipei, Taiwan.

Cell lines and construction of cDNA expression vectors, stable gene transfection, and transgene studies

The mouse B16F10 (B16) melanoma cell line was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). B16 cells stably transfected with hgp100 cDNA vector, designated as B16-hgp100, were obtained as reported previously.[13] pNiFty-secreted embryonic alkaline phosphatase (SEAP) plasmid was purchased from InvivoGen (San Diego, CA, USA). The hgp100 cDNA expression plasmid pWRG1644 was constructed as reported previously.[14]

Transfection and SEAP reporter assay

B16-hgp100 melanoma cells were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2/95% air incubator. The cells were seeded as 2×10^4 cells/well in a 96-well plate. After 24 h, B16-hgp100 melanoma cells were transfected with pNiFty-SEAP plasmid in the presence of lipofectin for 24 h.