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Phenethyl Isothiocyanate Inhibits *In Vivo* Growth of Subcutaneous Xenograft Tumors of Human Malignant Melanoma A375.S2 Cells

WEI-YA NI[1*], HSU-FENG LU[2*], SHU-CHUN HSU[3], YU-PING HSIAO[4,5], KUO-CHING LIU[6], JIA-YOU LIU[2], BIN-CHUAN JI[7], SHU-CHING HSUEH[2], FANG-MING HUNG[8], HUNG-SHENG SHANG[9*] and JING-GUNG CHUNG[3,10]

[1]Department of Food and Beverage Management, NanKai University of Technology, Nantou County, Taiwan, R.O.C.;
Departments of [2]Clinical Pathology, Cheng Hsin General Hospital, Taipei, Taiwan, R.O.C.;
Departments of [3]Biological Science and Technology and [6]Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan, R.O.C.;
[4]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, R.O.C.;
[5]Department of Dermatology, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.;
[7]Division of Chest Medicine, Department of Internal Medicine, Changhua Christian Hospital, Changhua, Taiwan, R.O.C.;
[8]Department of Surgical Intensive Care Unit, Far Eastern Memorial Hospital, Taipei, Taiwan, R.O.C.;
[9]Department of Pathology, National Defense Medical Center, Division of Clinical Pathology, Tri-Service General Hospital, Taipei, Taiwan, R.O.C.;
[10]Department of Biotechnology, Asia University, Taichung, Taiwan, R.O.C.

Abstract. *Numerous studies have shown that phenethyl isothiocyanate (PEITC) induces apoptosis of different types of human cancer cell lines, however, there are no reports showing that PEITC inhibits tumor growth in a xenograft model of melanoma in nude mice. We investigated effects of PEITC on the growth of xenografted A375.S2 cell tumors in nude BALB/c mice. A375.S2 cancer cells were inoculated subcutaneously into the lower flanks of mice. Seven days post-inoculation, mice having one palpable tumor were randomly divided into three groups and injected intraperitoneally with PEITC (0, 20 and 40 mg/kg). PEITC reduced tumor weight but total body weight was unaffected. These in vivo results provide support for further investigations to determine the potential use of PEITC as an anticancer drug.*

*These Authors contributed equally to this study.

Correspondence to: Jing-Gung Chung, Ph.D., Department of Biological Science and Technology, China Medical University. No 91, Hsueh-Shih Road, Taichung 404, Taiwan. Tel: +886 422053366 ext. 2161, Fax: +886 422053764, e-mail: jgchung@mail.cmu.edu.tw and Dr. Hung-Sheng Shang, Department of Pathology, National Defense Medical Center, Division of Clinical Pathology, Tri-Service General Hospital, No 325, Sec 2, Cheng-Kung Rd. Nei-Hu, Taipei, Taiwan. E-mail: iamkeith001@yahoo.com.tw

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Melanoma is becoming more common, increasing by approximately 4% annually (1) and resulting in increased mortality, especially in Western countries (2). Melanoma accounts for 4% of all skin cancer worldwide yet it causes the greatest number of skin cancer-related deaths (3). The current treatments for melanoma or other types of skin cancer are surgery, radiation, chemotherapy, or various combinations, but mortality remains high (4). New approaches to treating cancer are required, including identifying potential anticancer compounds from natural products.

Dietary intake of cruciferous vegetables (broccoli, cabbage, watercress, may provide protection against different diseases, including cancer (5-7). Cruciferous vegetables contain organic isothiocyanates which have been shown to reduce the development of various malignancies (8) and to inhibit cancer formation (9). Phenethyl isothiocyanate (PEITC) is one of these compounds and has cancer chemopreventive activity (10).

PEITC induced apoptosis of lung cancer (11), leukemia (12), colon cancer (13), breast cancer (14) prostate cancer (15), osteogenic sarcoma (16), ovarian cancer (17) and oral

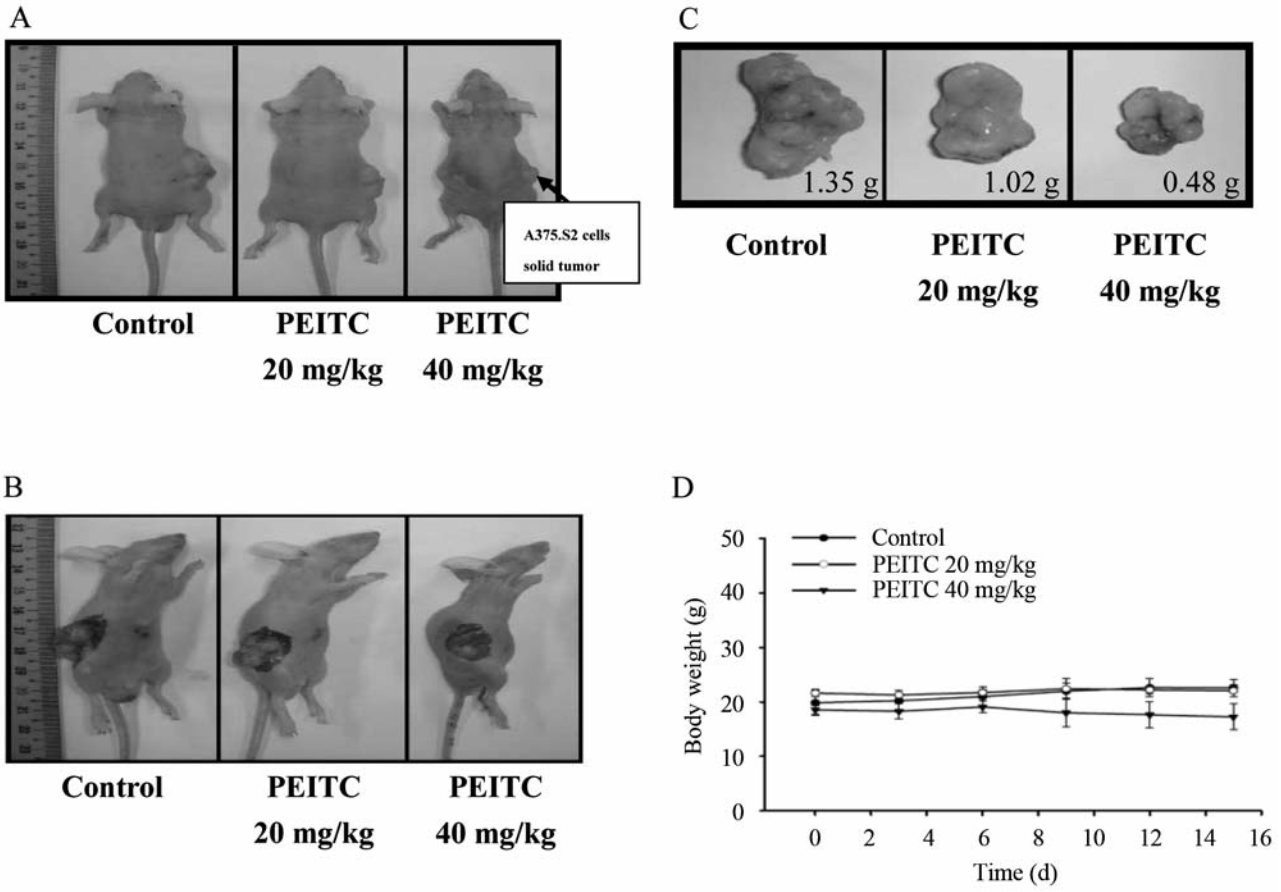

Figure 1. *Phenethyl Isothiocyanate (PEITC) affects the growth of subcutaneously implanted A375.S2 cells on BALB/c nude mice in vivo. For subcutaneous tumor growth study, A375.S2 cancer cells (1×10^6 cells in 0.1 ml Dulbecco's PBS) were inoculated subcutaneously into the lower flanks of each mouse. Seven days after cancer cell inoculation, each mouse had produced one palpable tumor and mice were then randomly divided into three groups (n=10/group). One group of mice were given intraperitoneal injections of the vehicle (olive oil) only; another group were given intraperitoneal injections of vehicle containing 20 mg/kg PEITC, and a final group were given intraperitoneal injections of vehicle with 40 mg/kg PEITC. All the mice were monitored weekly for tumor growth and were treated at the above doses daily for up to 12 days before being weighed as described in the Materials and Methods. Representative mice (A), representative tumors (B), tumor weight (C), total body weight (D) are shown.*

squamous carcinoma (18) cell lines. Novel combinations, such as metformin and PEITC, show promise in expanding ovarian cancer therapies and overcoming the high incidence of cisplatin-resistant cancer (19). The combination of PEITC and taxol exhibited a synergistic effect on growth inhibition of breast cancer cells (20). PEITC had anti-angiogenic effects in an animal model of chemically-induced breast cancer (21). Recently, it was reported that PEITC induced expression of damaged DNA-binding protein 2 (DDB2), and that expression of DDB2 was critical for effective response of tumors to PEITC (22). There are several studies showing that PEITC induced cytotoxic effects in different cancer cell lines, however, there are no reports on the effects of PEITC in a mouse xenograft model using human melanoma cells. The present study determined if PEITC would inhibit tumor growth in a xenograft mouse model of human melanoma.

Materials and Methods

Chemicals and reagents. PEITC and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Minimum essential medium (MEM), L-glutamine, fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY, USA).

Cell culture. The human melanoma cancer cell line (A375.S2) was purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in MEM supplemented with 10% FBS, 1% antibiotics (100 Units/ml penicillin and 100 μg/ml streptomycin) and 2 mM L-glutamine at 37 °C in an incubator with humidified 5% CO_2 and 95% air at one atmosphere. The medium was changed every two days (18, 23, 24).

Subcutaneous implantation of A375.S2 cells and PEITC treatment. Thirty male BALB/c nude mice, six weeks old with a body weight

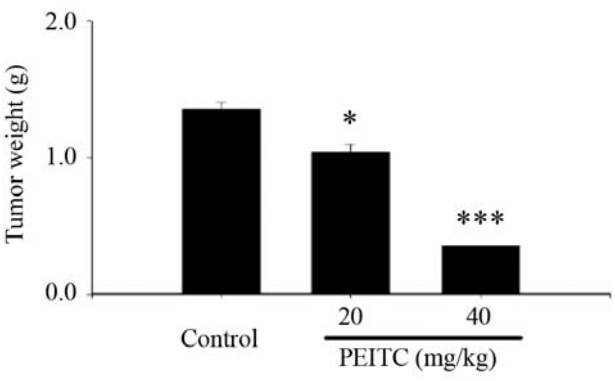

Figure 2. *Tumor weight of xenograft of A375.S2 cells in BALB/c nude mice were treated with Phenethyl Isothiocyanate (PEITC). Significantly different from the control at *p<0.05 and ***p<0.001.*

of approximately 25 g weight, were purchased from the National Laboratory Animal Center (Taipei, Taiwan). Animals were maintained in the Laboratory Animal Center of China Medical University and cared for as required by the animal guidelines (Affidavit of Approval of Animal Use Protocol) of China Medical University. For subcutaneous tumor growth study, A375.S2 cancer cells (1×10^6 cells in 0.1 ml Dulbecco's PBS) were inoculated subcutaneously into the lower flanks of mice. Seven days after the inoculation, mice having one palpable tumor were randomly assigned to one of three groups (n=10/group). Group I mice received intraperitoneal (*i.p.*) injections of the vehicle (olive oil) for 12 days. Group II mice were injected *i.p.* with 20 mg/kg PEITC for 12 days. Group III mice received *i.p.* injections of 40 mg/kg PEITC for 12 days. Mice were monitored weekly for tumor growth (25).

Statistical analysis. Data are presented as mean±standard deviation (S.D.). Student's *t*-test was used to compare the difference between control and PEITC treatments in each group. A *p*-value of less than 0.05 was considered statistically significant.

Results

This study determined effects of PEITC on tumor growth *in vivo*, of human melanoma cells injected into BALB/c nude mice. Xenograft A375.S2 tumors were allowed to grow for one week before being treated with PEITC (20 or 40 mg/kg/mouse). Data in Figure 1 show that PEITC did not alter total body weight of mice. PEITC significantly reduced tumor weight as seen in Figure 2. This effect was dose-dependent with 40 mg/kg having a larger effect compared to 20 mg/kg.

Discussion

Several studies have reported that PEITC induced cytotoxicity effects in human cancer cell lines (16, 18, 24, 26-28). We have previously shown that PEITC induced apoptosis in human oral squamous carcinoma HSC-3 cells and osteogenic sarcoma U2OS cells. (16, 18, 29). However, there are no reports showing effects of PEITC on A375.S2 cell xenograft tumors in mice. We report for the first time that PEITC reduced the weight and size of A375.S2 cell tumors in nude mice. We have earlier reported that PEITC induced cell-cycle arrest in A375.S2 cells (24). It is well-documented that cell-cycle control represents a major regulatory mechanism of cell growth (29).

Numerous studies have shown that natural products possess antitumor activity in various human cancer cell lines *in vitro* and in xenograft systems of human cancer cell tumors *in vivo*. Several anticancer drugs used clinically are derived from plants. Taxol, for example, has widespread use in treating lung, ovary and breast cancer and has been shown to induce (30) apoptosis in various human cancer cell lines (31). Another clinically-used drug derived from a plant is genistein, used for treating breast cancer (32), it also induces apoptosis (33).

We have shown in earlier *in vitro* studies that PEITC induced apoptosis of human prostate cancer DU 145 (28), osteogenic sarcoma U2OS (16), colon cancer HT29 (26) and oral cancer HSC-3 (18) cell lines. We also found that PEITC can promote immune responses in normal and WEHI-3 leukemic mice *in vivo* (34). Those findings raised the possibility PEITC may have potential as an anticancer drug. We now show that in a dose-dependent manner, PEITC reduced tumor size and weight in A375.S2 xenograft tumors in nude mice. These *in vivo* results provide support for further investigations to determine the potential use of PEITC as an anticancer drug.

Conflicts of Interest

The Authors declare no conflict of interest.

Acknowledgements

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