

Suppressing Effects of Dietary Supplementation of the Organoselenium 1,4-Phenylenebis(methylene)selenocyanate and the *Citrus* Antioxidant Auraptene on Lung Metastasis of Melanoma Cells in Mice¹

Takuji Tanaka,² Hiroyuki Kohno, Manabu Murakami, Seiko Kagami, and Karam El-Bayoumy

Departments of Pathology [T. T., S. K.] and Serology [H. K.], Division of Basic Science, Medical Research Institute [M. M.], Kanazawa Medical University, Ishikawa 920-0293, Japan, and Division of Cancer Etiology and Prevention, American Health Foundation, Valhalla, New York 10595 [K. E.-B.]

Abstract

The modifying effects of the organoselenium 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) and the *Citrus* antioxidant auraptene as dietary supplements on experimental pulmonary metastasis of B16BL6 murine melanoma cells were investigated in an i.v. injection model in mice. Seven groups of male C57BL/6 mice were fed a basal diet (control group) or the basal diet supplemented with *p*-XSC (4, 8, or 15 mg/kg) or auraptene (250, 500, or 1000 mg/kg). All mice were fed their respective diet for 2 weeks before and after i.v. injection of 1×10^5 viable melanoma cells. At termination of the study, the incidence of lung metastatic tumors was determined. Cross-sectional areas and tumor volumes were analyzed morphometrically. In addition, apoptotic indices of lung metastatic tumors of all groups were counted. The incidences of lung metastasis in mice fed the diet mixed with 8 or 15 mg *p*-XSC/kg were significantly smaller than that in mice fed the basal diet. The mean numbers of metastatic lung tumors were significantly lower in mice fed *p*-XSC (4, 8, and 15 mg/kg) and auraptene (500 and 1000 mg/kg) than in controls. Cross-sectional areas and volumes of the tumors were also significantly decreased in mice given *p*-XSC (8 or 15 mg/kg) and auraptene (500 mg/kg). Apoptotic indices in mice fed the diets mixed with *p*-XSC (4, 8, or 15 mg/kg) and auraptene (500 and 1000 mg/kg) were significantly greater than those in the control group. These results indicate that in mice, diet supplementation with *p*-XSC and auraptene reduces pulmonary metastasis of B16BL6 melanoma cells and inhibits the growth of these metastatic tumors in lung, in part, by inducing apoptosis. We suggest that these agents, especially *p*-XSC, may be valuable in preventing metastatic diseases in future studies in the clinic.

Introduction

Metastasis is the most devastating aspect of malignant neoplasms. Although advances in surgery, chemotherapy, and radiotherapy have significantly improved the treatment of primary malignancies, the occurrence of metastasis still leads to poor prognosis and death in patients with malignancy. A complex series of steps is required to permit the successful establishment of tumor metastasis (1, 2). Several attempts to find antimetastasis agents have been made using animal experimental metastasis models. In many experiments, attempts have been made to model some of the postintrasavasation events in hematogenous metastasis by giving rodents a single intravascular injection of cancer cells. However, metastasis is a continuous process; therefore, the fate of successive waves of cancer cells arriving in the same

organ is important. In this context, an experimental metastasis mouse model using B16BL6 melanoma cells (3, 4) is useful to identify possible antimetastasis agents (5–14).

A number of cancer chemopreventive agents effectively inhibit carcinogenesis and lower the incidence of cancer development in laboratory animal model assays (15, 16). Studies on the antimetastatic effects of chemopreventive compounds are limited but include reports on compounds that have chemopreventive effects in chemical carcinogenesis and also have antimetastatic potential (9–14). The antioxidant *N*-acetylcysteine (3) has recently been found to inhibit neoangiogenesis by blocking endothelial cell invasion, metalloproteinase production, activation, and degradation of substrate (17). This suggests a possible antimetastatic effect (18) similar to that observed with lecitinized ascorbic acid in experimental metastasis (6).

We have previously found that a *Citrus* auraptene (Fig. 1; Refs. 19–21) and the synthetic organoselenium compound *p*-XSC³ (Fig. 1; Refs. 22 and 23) suppress chemical carcinogenesis in rodents. An interesting observation regarding the role of selenium on metastasis is that an inverse relationship between serum selenium level and the rate of distant metastases in cancer patients has been reported (24, 25). These compounds exert chemopreventive effects through modification of cell proliferation and/or the activity of detoxifying enzymes, induction of detoxifying enzyme activity, and/or induction of apoptosis (19–23, 26).

Apoptosis, or programmed cell death, is an active physiological mode of cell death in which the cell dies by a programmed cell process (27). Apoptosis seems to be the most common morphology when cell death is physiologically determined. During apoptosis, nuclear condensation (forming peripheral chromatin cap) and fragmentation of nuclei occur. The cell shrinks due to loss of cytoplasmic volume and condensation of cytoplasmic protein. Thus, blebbing occurs and fragments cellular components into intact membrane-bound bodies. These so-called apoptotic bodies are rapidly phagocytosed by neighboring cells (28). There are reports on the induction of apoptosis in primary or metastatic malignant neoplasms that also describe an inhibition of metastasis (8, 29–32). Genistein, a candidate cancer chemopreventive agent, also induces apoptosis and may inhibit metastasis (8).

In the present study, the modifying effects of dietary *p*-XSC and auraptene on experimental metastasis of melanoma cells were studied in a model assay in mice that had been injected with viable B16BL6 melanoma cells, which are syngeneic to C57BL/6 mice (33). The effects of both compounds on apoptosis in metastatic melanoma cells in the lungs were also examined.

Received 1/17/00; accepted 5/24/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported in part by Comprehensive Research Project on Health Sciences Focusing on Drug Innovation Grant HS-52260 from the Japan Health Sciences Foundation, Grants C99-1 and P99-1 from Kanazawa Medical University, and National Cancer Institute Grant PO1-70972.

² To whom requests for reprints should be addressed, at Department of Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan. E-mail: takutt@kanazawa-med.ac.jp.

³ The abbreviations used are: *p*-XSC, 1,4-phenylenebis(methylene)selenocyanate; AI, apoptotic index.

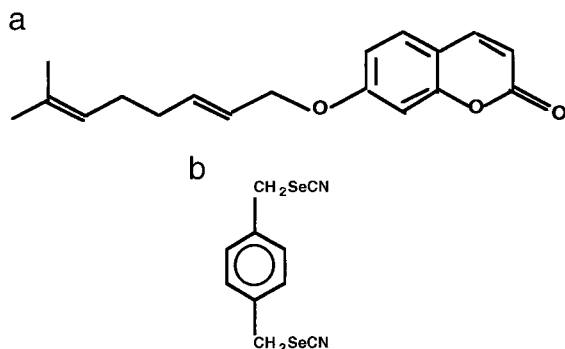


Fig. 1. Chemical structures of (a) auraptene and (b) *p*-XSC.

Materials and Methods

Animals, Diets, and Chemicals. Seven-week-old male C57BL/6 mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were housed five mice/box in wire-topped plastic boxes in a laboratory that was maintained on a 12-h light/12-h dark cycle. The temperature and humidity in the room were controlled at $23 \pm 2^\circ\text{C}$ and $50 \pm 10\%$, respectively. Powdered CE-2 (CLEA Japan, Inc., Tokyo, Japan) was used as a basal diet. Dr. Akira Murakami (Department of Biotechnological Science, Faculty of Biology-oriented Science and Technology, Kinki University, Wakayama, Japan) provided auraptene isolated from *natsumikans* (*Citrus natsumidai hayata*) in Wakayama Prefecture (Japan). *p*-XSC (>99% pure) was synthesized as described previously (34). Experimental diets mixed with *p*-XSC [4 mg/kg (2 mg as selenium), 8 mg/kg (4 mg as selenium), and 15 mg/kg (7.5 mg as selenium)] or auraptene (250, 500, or 1000 mg/kg) were freshly prepared each week, and a feeder was placed in each cage. The diets were stored in a cold room (-4°C) until use. Both the experimental diets and deionized water were offered *ad libitum*. The amount of selenium in the basal diet was 0.2 mg/kg. There was no detectable selenium in the deionized water.

Cell Line. The B16BL6 murine melanoma cell line was obtained from the Cancer Research Institute, Kanazawa University (Kanazawa, Japan). The cells were grown in a 3:7 mixture of Ham's F10 and L-15 containing 10% fetal bovine serum (Medical & Biological Laboratories, Co., Ltd., Nagoya, Japan), penicillin (50 units/ml), and streptomycin (50 $\mu\text{g}/\text{ml}$). Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C .

Experimental Procedure. A total of 72 mice were fed the basal diet for 7 days before being randomly assigned to one of seven groups of 10 or 11 mice each; they were then fed the basal diet, the basal diet mixed with *p*-XSC (4, 8, or 15 mg/kg), or the basal diet mixed with auraptene (250, 500, or 1000 mg/kg) for 2 weeks. The B16BL6 cells were harvested with 0.02% EDTA in HBSS and washed once with 2% fetal bovine serum-containing medium and twice with HBSS. Viability of the melanoma cells was determined with trypan blue (99% viability), and a single cell suspension was made in HBSS. Each mouse was injected with 1×10^5 viable cells in a total volume of 0.2 ml via the lateral

tail vein. The mice were then maintained on the diet for another 2 weeks. On termination, mice were killed by cervical dislocation; their lungs and livers were excised and thoroughly rinsed with PBS to remove residual blood, fixed in 10% buffered formalin, and analyzed for metastatic tumors.

Morphometric Analysis of Metastatic Tumors. The numbers of pulmonary and liver metastatic tumors were determined by counting the black foci in both organs under a dissecting microscope (MZ FL III; Leica Co., Inc., Tokyo, Japan). The incidence of metastases was calculated by dividing the number of mice with metastatic tumors by the total number of mice in each group. The cross-sectional areas of metastatic tumors in randomly selected fields from the lungs of each tumor-bearing mouse were measured using an image analysis system (Q500 IW; Leica Co., Inc.). The metastatic tumor volume was calculated based on the longest diameter measured and on the assumption that the tumors were spherical (35). The contrast between the black metastatic melanoma tumors and the natural color of the lungs makes the lesions beneath the lung surface readily detectable with the image analysis system. Thus, the number of tumors in the lungs represents the total number of visible tumors. A few liver metastases were present (see "Results"), and they were analyzed in a manner similar to that described above.

Determination of the AI. Apoptotic cells were detected in H&E-stained sections of metastatic lung tumors under a light microscope with high-power magnification ($\times 400$), according to criteria of Kerr *et al.* (27) and Walker *et al.* (36). The AI was expressed as the percentage of apoptotic cells and apoptotic bodies relative to all tumors cells of the metastatic lesion and was calculated after counting at least 500 cells of 20 metastatic lung lesions of each animal.

Statistics. Fisher's exact probability test, χ^2 test, unpaired Student's *t* test, or Welch's *t* test was used for statistical analyses. A value of $P < 0.05$ was considered significant.

Results

General Observations. Dietary administration of test compounds did not show any adverse effect on the growth of mice during the study. The mean body weight of all mice at the start was 16.5 ± 2.0 g. As shown in Table 1, there were no significant differences in body weight gains among the groups throughout the study. Also, there were no significant differences on the mean food intake among the groups (Table 1).

Incidence of Metastasis. Several metastases were found in lungs in all dietary groups. However, a few liver metastases were present in two groups: (a) 20% (2 of 10 mice) of mice in group 1 (untreated); and (b) 9% (1 of 11 mice) of mice in group 4 (1000 mg auraptene/kg). The incidence and the numbers of metastatic lung tumors are indicated in Table 1. The incidence rates of lung metastasis in groups 1–4 were 100%. However, the frequency of lung metastasis in groups 5 (80%), 6 (60%), and 7 (45%) was lower than that in group 1. The incidence rates in groups 6 and 7 were significantly smaller than those in group 1 ($P = 0.0433$ or $P = 0.0085$, respectively). The mean number of lung metastases in group 1 was 83 ± 16 . The number of

Table 1 Effects of dietary auraptene and *p*-XSC on the incidence of lung metastasis of B16BL6 melanoma cells in male C57BL/6 mice

Group no.	Treatment (no. of mice examined)	Body weight (g)	Food intake (g/mouse/day)	No. of mice with lung metastatic tumors				Incidence of lung metastasis	No. of metastatic lung tumors/mouse
				0 tumor	1–50 tumors	51–100 tumors	≥ 101 tumors		
1	None (11)	23.4 ± 1.9^a	1.36 ± 0.30	0	2	8	1	100% (11/11)	83 ± 16
2	Auraptene, 250 mg/kg (10)	22.9 ± 1.8	1.37 ± 0.32	0	1	8	1	100% (10/10)	73 ± 28
3	Auraptene, 500 mg/kg (10)	23.7 ± 2.5	1.36 ± 0.26	0	4	5	1	100% (10/10)	62 ± 24^b
4	Auraptene, 1000 mg/kg (10)	23.8 ± 1.9	1.40 ± 0.41	0	4	5	1	100% (10/10)	58 ± 27^c
5	<i>p</i> -XSC, 4 mg/kg, 2 mg as selenium (10)	23.5 ± 1.8	1.41 ± 0.35	2	4	4	0	80% (8/10)	51 ± 32^d
6	<i>p</i> -XSC, 8 mg/kg, 4 mg as selenium (10)	23.3 ± 1.5	1.40 ± 0.38	4	4	2	0	60% ^d (6/10)	34 ± 36^e
7	<i>p</i> -XSC, 15 mg/kg, 7.5 mg as selenium (11)	22.8 ± 1.2	1.38 ± 0.29	6	2	3	0	45% ^f (5/11)	24 ± 29^g

^a Mean \pm SD.

^b Significantly different from group 1 by Student's *t* test or Welch's *t* test ($P < 0.05$).

^c Significantly different from group 1 by Student's *t* test or Welch's *t* test ($P < 0.02$).

^d Significantly different from group 1 by Fisher's exact probability test. ($P = 0.0433$).

^e Significantly different from group 1 by Student's *t* test or Welch's *t* test ($P < 0.002$).

^f Significantly different from group 1 by Fisher's exact probability test ($P = 0.0085$).

^g Significantly different from group 1 by Student's *t* test or Welch's *t* test ($P < 0.001$).

Table 2 Effects of dietary auraptene and *p*-XSC on the area and volume of lung metastatic tumors of B16BL6 melanoma cells in male C57BL/6 mice

Group no.	Treatment (no. of mice examined)	Area of metastatic lung tumors on cross section (mm ²)	Volume of metastatic lung tumors (mm ³)
1	None (11)	0.28 ± 0.04 ^a	0.20 ± 0.04
2	Auraptene, 250 mg/kg (10)	0.28 ± 0.02	0.21 ± 0.02
3	Auraptene, 500 mg/kg (10)	0.24 ± 0.01 ^b	0.16 ± 0.01 ^b
4	Auraptene, 1000 mg/kg (10)	0.25 ± 0.02	0.18 ± 0.02
5	<i>p</i> -XSC, 4 mg/kg, 2 mg as selenium (10)	0.24 ± 0.01 ^b	0.20 ± 0.02
6	<i>p</i> -XSC, 8 mg/kg, 4 mg as selenium (10)	0.18 ± 0.01 ^c	0.13 ± 0.01 ^c
7	<i>p</i> -XSC, 15 mg/kg, 7.5 mg as selenium (11)	0.09 ± 0.01 ^c	0.12 ± 0.01 ^c

^a Mean ± SD.^b Significantly different from group 1 by Welch's *t* test ($P < 0.02$).^c Significantly different from group 1 by Welch's *t* test ($P < 0.001$).

metastatic tumors in groups 3 (62 ± 2), 4 (58 ± 27), 5 (51 ± 32), 6 (34 ± 36), and 7 (24 ± 29) was significantly lower than that in group 1 ($P < 0.05$ vs. group 3, $P < 0.02$ vs. groups 4 and 5, $P < 0.002$ vs. group 6, or $P < 0.001$ vs. group 7).

Morphometric Analysis of Lung Metastatic Lesions. The data on morphometric analysis of lung metastatic tumors are summarized in Table 2. The mean areas of tumor cross-section in groups 3 (0.24 ± 0.01), 5 (0.24 ± 0.01), 6 (0.18 ± 0.01), and 7 (0.09 ± 0.01) were significantly decreased compared with those in group 1 (0.28 ± 0.04 ; $P < 0.02$ vs. groups 3 and 5 or $P < 0.001$ vs. groups 6 and 7). Also, the mean volumes of lung metastases in groups 3 (0.16 ± 0.01), 6 (0.13 ± 0.01), and 7 (0.12 ± 0.01) were significantly smaller than those in group 1 (0.20 ± 0.04 ; $P < 0.02$ vs. group 3 or $P < 0.001$ vs. groups 6 and 7).

AI Index. Apoptotic tumor cells were found randomly and singly in the metastatic tumors in lungs. The mean AI value of group 1 was $3.8 \pm 1.2\%$ (see Fig. 2). The mean AIs of groups 3 ($4.8 \pm 1.0\%$), 4 ($5.8 \pm 2.8\%$), 5 ($6.8 \pm 1.6\%$), 6 ($7.1 \pm 1.4\%$), and 7 ($8.6 \pm 3.4\%$) were significantly lower than those in group 1 ($P < 0.01$ vs. groups 3 and 4 or $P < 0.001$ vs. groups 5, 6, and 7).

Discussion

In the present study, dietary supplementation suppressed experimental metastasis of B16BL6 melanoma cells in mice, as revealed by the low incidence of metastases in the lungs, the small number of metastatic lesions, and the low volume of metastatic lesions in mice fed the diets mixed with *p*-XSC and auraptene compared with those in mice fed the basal diet. It should be noted that the inhibition by both compounds was dose dependent and that *p*-XSC was more potent than auraptene. Treatment with test compounds did not induce weight loss in tumor-bearing mice. Our findings suggest that the antimetastatic effects of auraptene and *p*-XSC were demonstrated in the absence of side effects such as weight loss, which might indirectly affect metastasis. Thus, the results in the current study provide the first evidence that dietary *p*-XSC and auraptene reduce experimental metastasis.

Other forms of selenium, such as selenite and selenomethionine, have been reported to be inhibitors of lung metastasis in laboratory animals (12, 14). However, the fact that rodents can tolerate dietary *p*-XSC much better than selenite and selenomethionine (22) suggests that *p*-XSC would be the more likely candidate for use in future clinical trials.

Active oxygen radicals are known to be involved in the development of various chronic diseases, including cancer. Therefore, antioxidants such as auraptene have been effective as antitumor agents. Saintot *et al.* (37) reported that the presence of nodes and/or metastases is directly associated with low plasma concentrations of cholesterol and malondialdehyde. Auraptene likely reduces the production of lipid peroxidation products, including malondialdehyde, in rat

carcinogenesis (21). Thus, we would expect that the antioxidative property of auraptene contributes to the antimetastatic activity found in the present study. In addition, the immunostimulatory action of auraptene (38) could be one of the mechanisms of antimetastatic action (18).

The process of tumor metastasis is very complex and is made up of many biological events (reviewed in Refs. 2 and 39). Tumor cells must detach from the primary lesion, invade the surrounding extracellular matrix, and intravasate into the bloodstream, where they must dodge the host's immune system until they reach the secondary site. At the secondary site, the tumor cells adhere to endothelial cells and invade through the endothelium into the matrix (extravasation). Finally, the tumor cells begin to grow at the secondary site. Interventions that block any of these steps can theoretically prevent the spread of malignant cells. In this study, metastatic potential was evaluated after direct injection of B16BL6 melanoma cells into the bloodstream via the tail vein. This model eliminates intravasation but is able to measure the ability of malignant cells to extravasate into the lungs. We found that dietary administration of *p*-XSC and auraptene decreased the number of metastatic tumors that developed in the lungs. This suggests that *p*-XSC and auraptene affect extravasation of malignant cells. It is of interest to investigate the effects of *p*-XSC and auraptene on an early step of metastasis (*i.e.*, the ability of malignant cells to release from the primary lesion and invade the blood vessels).

The efficiency of various antitumor agents is related to the intrinsic ability of the target tumor cells to respond to these agents by inducing apoptosis (40). Induction of apoptosis and inhibition of neoangiogenesis in primary and/or metastatic tumors were shown to inhibit tumor metastasis (8, 29–32). In the present study, auraptene and *p*-XSC induced apoptosis in metastatic lung tumors. Some chemopreventive antioxidants such as ascorbic acid and *N*-acetylcysteine were reported to induce apoptosis (41). Induction of apoptosis by *p*-XSC is also a likely mechanism for the inhibition of carcinogenesis (26). In fact, there are several reports referring to apoptosis as one of the mechanisms of organoselenium-induced tumor inhibition (26). *p*-XSC is a more potent inducer of apoptosis than selenite in a mammary carcinoma cell line (42, 43). The primary metabolite of selenite, selenodiglutathione, also induces apoptosis in mouse erythroleukemia cells (44). Thus, induction of apoptosis may play a critical role in suppression of lung metastasis of melanoma cells by auraptene and *p*-XSC. Additional studies are required to delineate the effects of *p*-XSC and auraptene on neoangiogenesis (45) and on the expression of matrix metalloproteinases (46), integrins (47), nitric oxidase (48, 49), and

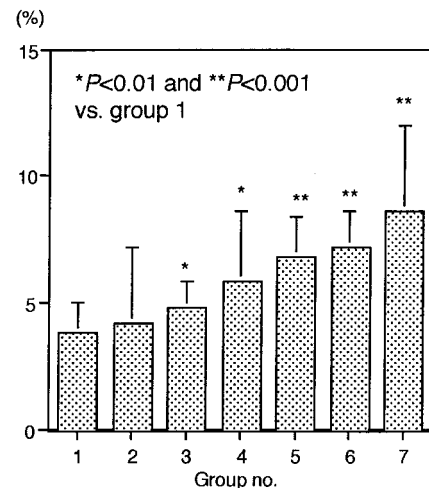


Fig. 2. AI of lung metastatic tumors in each group.

metastasis-suppressing gene product (31). These studies are under way in our laboratories. The elucidation of the mechanisms through which the compounds, especially *p*-XSC, exert their antimetastatic effects represents a fascinating aspect of research in the oncological field.

References

- Fidler, I. The evolution of biological heterogeneity in metastatic neoplasms. In: G. L. Nicolson and L. Milas (eds.), *Cancer Invasion and Metastases: Biologic and Therapeutic Aspects*, pp. 5–26. New York: Raven Press, 1984.
- Fidler, I. J. Critical determinants of cancer metastasis: rationale for therapy. *Cancer Chemother. Pharmacol.*, **43** (Suppl.): S3–S10, 1999.
- Fidler, I. J. General considerations for studies of experimental cancer metastasis. *Methods Cancer Res.*, **15**: 399–439, 1978.
- Fidler, I. J., Gersten, D., and Hart, I. R. The biology of cancer, invasion and metastasis. *Adv. Cancer Res.*, **28**: 149–249, 1978.
- Yudoh, K., Matsuno, H., and Kimura, T. $1\alpha,25$ -Dihydroxyvitamin D₃ inhibits *in vitro* invasiveness through the extracellular matrix and *in vivo* pulmonary metastasis of B16 mouse melanoma. *J. Lab. Clin. Med.*, **133**: 120–128, 1999.
- Takenaga, M., Igarashi, R., Nakayama, T., and Mizushima, Y. Lecithinized ascorbic acid (PC-AS) effectively inhibits murine pulmonary metastasis. *Anticancer Res.*, **19**: 1085–1091, 1999.
- Mishima, T., Murata, J., Toyoshima, M., Fujii, H., Nakajima, M., Hayashi, T., Kato, T., and Saiki, I. Inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Pirulina platensis*. *Clin. Exp. Metastasis*, **16**: 541–550, 1998.
- Li, Y., Bhuiyan, M., and Sarkar, F. H. Induction of apoptosis and inhibition of c-erbB-2 in MDA-MB-435 cells by genistein. *Int. J. Oncol.*, **15**: 525–533, 1999.
- Li, D., Yee, J. A., McGuire, M. H., Murphy, P. A., and Yan, L. Soybean isoflavones reduce experimental metastasis in mice. *J. Nutr.*, **129**: 1075–1078, 1999.
- Li, D., Yee, J. A., Thompson, L. U., and Yan, L. Dietary supplementation with secoisolariciresinol diglycoside (SDG) reduces experimental metastasis of melanoma cells in mice. *Cancer Lett.*, **142**: 91–96, 1999.
- Liu, Y. H., Tian, H. S., and Wang, D. X. Inhibitory effect of selenium yeast on the metastasis of Lewis lung carcinoma in C57BL mice. *Chin. Med. J. (Engl. Ed.)*, **100**: 549–554, 1987.
- Yan, L., Yee, J. A., McGuire, M. H., and Graef, G. L. Effect of dietary supplementation of selenite on pulmonary metastasis of melanoma cells in mice. *Nutr. Cancer*, **28**: 165–169, 1997.
- Yan, L., Yee, J. A., Li, D., McGuire, M. H., and Thompson, L. U. Dietary flaxseed supplementation and experimental metastasis of melanoma cells in mice. *Cancer Lett.*, **124**: 181–186, 1998.
- Yan, L., Yee, J. A., Li, D., McGuire, M. H., and Graef, G. L. Dietary supplementation of selenomethionine reduces metastasis of melanoma cells in mice. *Anticancer Res.*, **19**: 1337–1342, 1999.
- Singh, D. K., and Lippman, S. M. Cancer chemoprevention. Part 1: retinoids and carotenoids and other classic antioxidants. *Oncology (Basel)*, **12**: 1643–1658, 1998.
- Singh, D. K., and Lippman, S. M. Cancer chemoprevention. Part 2: hormones, nonclassic antioxidant natural agents, NSAIDs, and other agents. *Oncology (Basel)*, **12**: 1787–1800, 1998.
- Cai, T., Fassina, G., Morini, M., Aluigi, M. G., Masiello, L., Fontanini, G., D'Agostini, F., De Flora, S., Noonan, D. M., and Albini, A. *N*-Acetylcysteine inhibits endothelial cell invasion and angiogenesis. *Lab. Invest.*, **79**: 1151–1159, 1999.
- McCarty, M. F. An antithrombotic role for nutritional antioxidants: implications for tumor metastasis and other pathologies. *Med. Hypotheses*, **19**: 345–357, 1986.
- Tanaka, T., Kawabata, K., Kakumoto, M., Makita, H., Hara, A., Mori, H., Staoh, K., Hara, A., Murakami, A., Kuki, W., Takahashi, Y., Yonei, H., Koshimizu, K., and Ohigashi, H. *Citrus auraptene* inhibits chemically induced colonic aberrant crypt foci in male F344 rats. *Carcinogenesis (Lond.)*, **18**: 2155–2161, 1997.
- Tanaka, T., Kawabata, K., Kakumoto, M., Matsunaga, K., Mori, H., Murakami, A., Kuki, W., Takahashi, Y., Yonei, H., Staoh, K., Hara, A., Maeda, M., Ota, T., Odashima, S., Koshimizu, K., and Ohigashi, H. Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by *Citrus auraptene* in rats. *Carcinogenesis (Lond.)*, **19**: 425–431, 1998.
- Tanaka, T., Kawabata, K., Kakumoto, M., Hara, A., Murakami, A., Kuki, W., Takahashi, Y., Yonei, H., Maeda, M., Ota, T., Odashima, S., Yamane, T., Koshimizu, K., and Ohigashi, H. *Citrus auraptene* exerts dose-dependent chemopreventive activity in rat large bowel tumorigenesis: the inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of phase II drug-metabolizing enzymes. *Cancer Res.*, **58**: 2550–2556, 1998.
- El-Bayoumy, K., Upadhyaya, P., Chae, Y.-H., Sohn, O.-S., Rao, C. V., Fiala, E., and Reddy, B. S. Chemoprevention of cancer by organoselenium compounds. *J. Cell. Biochem.*, **22** (Suppl.): 92–100, 1995.
- Tanaka, T., Makita, H., Kawabata, K., Mori, H., and El-Bayoumy, K. 1,4-Phenylenebis(methylene)selenocyanate exerts exceptional chemopreventive activity in rat tongue carcinogenesis. *Cancer Res.*, **57**: 3644–3648, 1997.
- Brogamer, W. L. J., McConnel, K. P., and Blotky, A. L. Relationship between serum selenium levels and patients with carcinoma. *Cancer (Phila.)*, **37**: 1384–1388, 1976.
- Lockwood, K., Moesgaard, S., Hanioka, T., and Folkers, K. Apparent partial remission of breast cancer in “high risk” patients supplemented with nutritional antioxidants, essential fatty acids and coenzyme Q₁₀. *Mol. Aspects Med.*, **15**: s231–s240, 1994.
- Samaha, H. S., Hamid, R., El-Bayoumy, K., Rao, C. V., and Reddy, B. S. The role of apoptosis in the modulation of colon carcinogenesis by dietary fat and by the organoselenium compound 1,4-phenylenebis(methylene)selenocyanate. *Cancer Epidemiol. Biomark. Prev.*, **6**: 699–704, 1997.
- Kerr, J. F., Wyllie, A. H., and Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer*, **26**: 239–257, 1972.
- Kerr, J. F., Searle, J., Harmon, B. V., and Bishop, C. J. Apoptosis. In: C. S. Potten (eds.), *Perspectives on Mammalian Cell Death*, pp. 93–128. New York: Oxford University Press, 1987.
- Sakukawa, R., Murakami, K., Ikeda, T., Yamada, Y., and Saiki, I. Effect of 4-[3,5-bis(trimethylsilyl)benzamido]benzoic acid (TAC-101) on the liver metastasis of colon 26-L5 carcinoma cells. *Oncol. Res.*, **10**: 287–293, 1998.
- Baker, A. H., Georger, S. J., Zaltsman, A. B., Murphy, G., and Newby, A. C. Inhibition of invasion and induction of apoptotic cell death of cancer cell lines by overexpression of TIMP-3. *Br. J. Cancer*, **79**: 1347–1355, 1999.
- Ono, M., Handa, K., Withers, D. A., and Hakomori, S. Motility inhibition and apoptosis are induced by metastasis-suppressing gene product CD82 and its analogue CD9, with concurrent glycosylation. *Cancer Res.*, **59**: 2335–2339, 1999.
- Kamiya, K., Konno, H., Tanaka, T., Baba, M., Matsumoto, K., Sakaguchi, T., Yukita, A., Asano, M., Suzuki, H., Arai, T., and Nakamura, S. Antitumor effect on human gastric cancer and induction of apoptosis by vascular endothelial growth factor neutralizing antibody. *Jpn. J. Cancer Res.*, **90**: 794–800, 1999.
- Hart, I. R., Talmadge, J. E., and Fidler, I. J. Comparative studies on the quantitative analysis of experimental metastatic capacity. *Cancer Res.*, **43**: 400–402, 1983.
- El-Bayoumy, K., Chae, Y.-H., Upadhyaya, P., Meschter, C., Cohen, L. A., and Reddy, B. S. Selenium in chemoprevention of carcinogenesis. 11. Inhibition of 7,12-dimethylbenz(a)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague-Dawley rats by the synthetic organoselenium compound 1,4-phenylenebis(methylene)selenocyanate. *Cancer Res.*, **52**: 2402–2407, 1992.
- Welch, D. R., Neri, A., and Nicolson, G. L. Comparison of “spontaneous” and “experimental” metastasis using rat 13762 mammary adenocarcinoma metastatic cell clones. *Invasion Metastasis*, **3**: 65–80, 1983.
- Walker, N. I., Harmon, B. V., Gobe, G. C., and Kerr, J. F. Patterns of cell death. *Methods Achiev. Exp. Pathol.*, **13**: 18–54, 1988.
- Saintot, M., Astre, C., Pujol, H., and Gerber, M. Tumor progression and oxidant-antioxidant status. *Carcinogenesis (Lond.)*, **17**: 1267–1271, 1996.
- Tanaka, T., Sugiura, H., Inaba, R., Nishikawa, A., Murakami, A., Koshimizu, K., and Ohigashi, H. Immunomodulatory action of *Citrus auraptene* on macrophage functions and cytokine production of lymphocytes in female BALB/c mice. *Carcinogenesis (Lond.)*, **20**: 1471–1476, 1999.
- Hart, I. R., and Saini, A. Biology of tumour metastasis. *Lancet*, **339**: 1453–1457, 1992.
- Dive, C., and Hickmann, J. A. Drug-target interactions: only the first step in the commitment to a programmed cell death? *Br. J. Cancer*, **64**: 192–198, 1991.
- Sakagami, H., Satoh, K., Ohata, H., Takahashi, H., Yoshida, H., Iida, M., Kuribayashi, N., Sakagami, T., Momose, K., and Takeda, M. Relationship between ascorbyl radical density and apoptosis-inducing activity. *Anticancer Res.*, **16**: 2635–2644, 1996.
- Thompson, H., Wilson, A., Lu, J., Singh, M., Jiang, C., Upadhyaya, P., El-Bayoumy, K., and Ip, C. Comparison of the effects of an organic and an inorganic form of selenium on a mammary carcinoma cell line. *Carcinogenesis (Lond.)*, **15**: 183–186, 1994.
- Ronai, Z., Thompson, J. K., Traganos, P., Darzynkiewicz, Z., and Conaway, C. C. Effects of organic and inorganic selenium compounds on rat mammary tumor cells. *Int. J. Cancer*, **63**: 428–434, 1995.
- Lanfear, J., Fleming, J., Wu, L., Webster, G., and Harrison, P. R. The selenium metabolite selenodiglutathione induces p53 and apoptosis: relevance to the chemopreventive effects of selenium? *Carcinogenesis (Lond.)*, **15**: 1387–1392, 1994.
- Wu, J. Apoptosis and angiogenesis: two promising tumor markers in breast cancer. *Anticancer Res.*, **16**: 2233–2239, 1996.
- Sazuka, M., Imazawa, H., Shoji, Y., Mita, T., Hara, Y., and Isemura, M. Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. *Biosci. Biotechnol. Biochem.*, **61**: 1504–1506, 1997.
- Mizejewski, G. J. Role of integrins in cancer: survey of expression patterns. *Proc. Soc. Exp. Biol. Med.*, **222**: 124–138, 1999.
- Thomsen, L. L., and Miles, D. W. Role of nitric oxide in tumour progression: lessons from human tumours. *Cancer Metastasis Rev.*, **17**: 1107–1118, 1998.
- Yamamoto, T., Terada, N., Nishizawa, Y., Tanaka, H., Akedo, H., Seiyama, A., Shiga, T., and Kosaka, H. Effect of *N*^G-nitro-L-arginine and/or L-arginine on experimental pulmonary metastasis in mice. *Cancer Lett.*, **87**: 115–120, 1994.