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Rationale for the Use of Genistein-Containing Soy Matrices in Chemoprevention Trials for Breast and Prostate Cancer

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Abstract Pharmacologists have realized that tyrosine kinase inhibitors (TKI) have potential as anti-cancer agents, both in prevention and therapy protocols. Nonetheless, concern about the risk of toxicity caused by synthetic TKIs restricted their development as chemoprevention agents. However, a naturally occurring TKI (the isoflavone genistein) in soy was discovered in 1987. The concentration of genistein in most soy food materials ranges from 1–2 mg/g. Oriental populations, who have low rates of breast and prostate cancer, consume 20–80 mg of genistein/day, almost entirely derived from soy, whereas the dietary intake of genistein in the US is only 1–3 mg/day. Chronic use of genistein as a chemopreventive agent has an advantage over synthetic TKIs because it is naturally found in soy foods. It could be delivered either in a purified state as a pill (to high-risk, motivated patient groups), or in the form of soy foods or soy-containing foods. Delivery of genistein in soy foods is more economically viable ($1.50 for a daily dose of 50 mg) than purified material ($5/day) and would require no prior approval by the FDA. Accordingly, investigators at several different sites have begun or are planning chemoprevention trials using a soy beverage product based on SUPRO™, an isolated soy protein manufactured by Protein Technologies International of St. Louis, MO. These investigators are examining the effect of the soy beverage on surrogate intermediate endpoint biomarkers (SIEBs) in patients at risk for breast and colon cancer, defining potential SIEBs in patients at risk for prostate cancer, and determining whether the soy beverage reduces the incidence of cancer recurrence. These studies will provide the basis for formal Phase I, Phase II and Phase III clinical trials of genistein and soy food products such as SUPRO™ for cancer chemoprevention.

Key words: Animal models, delivery choice, genistein, mechanisms, soy

In 1994, a total of 400,000 new cases of breast and prostate cancer will be diagnosed in the US [1]. In addition, there will be 45,000 breast cancer deaths and 33,000 prostate cancer deaths. The death rate from these cancers when expressed per 100,000 of the American population has not changed over the past 60 years [2], despite improvements in diagnosis, treatment and therapy. The lifetime risk for breast cancer in women is one in nine; for men, prostate cancer risk is one in eleven [1].

Chemoprevention, as opposed to chemotherapy, has been underexplored as an approach to reduce the risk of and death from cancer. In order to systematically develop rational strategies in chemoprevention, investigators have attempted to determine the reasons for marked country-to-country variations in specific cancer incidences and mortality.

The peoples of Southeast Asia (*e.g.*, China, Indonesia, Japan, Korea, Singapore) have a four- to ten-fold lower incidence of and death from breast and prostate cancer [1]. However, following emigration to the US, the risk of these can-

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cers in Asian peoples rises rapidly in one generation to equal that of Americans [3]. Differences in the diet are thought to account for a large part of this variation [4,5].

The American diet's much higher fat content compared to the Asian diet was initially considered the most important factor leading to increased cancer risk in Americans [6], *i.e.*, suggesting that fat is *cancer-causing*. However, clinical trials performed in this country relating breast cancer risk to dietary fat intake have not supported this hypothesis [7,8]. A new hypothesis is being examined to determine whether one or more components of the Southeast Asian diet are *cancer-preventing*.

Asian diets contain a higher percentage of vegetable products. Soy is used to prepare soy milk, miso (used in soups and gravies), tofu (used in place of meat) and a variety of fermented products including soy paste and tempeh. The average intake of soy protein in Southeast Asia varies from 10 g/day in China to 30–50 g/day in Japan and Taiwan [9]. In contrast, Americans eat no more than 1–3 g/day. In a recent review of the epidemiological literature, Messina *et al.* [10] found that nearly two-thirds (16/27) of the reported studies associated soy intake with a reduction of cancer risk.

Whole soybeans and various soy foods have chemopreventive properties in animal models of cancer. Troll *et al.* [11] first reported that adding whole soybeans to the diet reduced the incidence of mammary tumors in female rats irradiated with X-rays. Barnes *et al.* [12] confirmed this observation in an *N*-methyl-*N*-nitrosourea (MNU)-induced model of breast cancer after first carefully controlling the diets so that they were isocaloric and isonitrogenous.

Several substances with chemopreventive properties have been identified in soy [13]. These include isoflavonoids, soybean protease inhibitors [see A. Kennedy and H. Manzone, this issue], phytosterols and saponins, phenolic acids and phytic acid.

This review summarizes the experimental evidence for soy isoflavonoids having a role in the prevention of cancer based on data obtained from animal models and studies in cell culture, and the mechanisms responsible. We conclude by considering the optimal way of delivering a chemopreventive agent that naturally occurs in an edible food.

ANIMAL MODELS OF CANCER

Soybeans contain large amounts (1–2 mg/g) of the isoflavones daidzein (4',7-dihydroxyisoflavone) and genistein (4',5,7-trihydroxyisoflavone) [14–16]. Most soy foods also contain high levels of isoflavones because of their binding to soy proteins. The exceptions are soy sauce and soy protein products which have been washed with aqueous alcohol during their processing [14]. Much of the data from animal models was obtained without knowledge of the isoflavone content of the soy material used [17]. Nonetheless, two-thirds of the reported studies found the soy food product either reduced the incidence of tumors, inhibited the rate at which tumors appeared, or reduced the total tumor number or tumor size. Interestingly, a soy protein concentrate which had been washed with aqueous alcohol (removing the isoflavones) had no effect on the appearance of mammary tumors in the 7,12-dimethylbenz[*a*]anthracene model of breast cancer. On the other hand, the aqueous alcohol extract, concentrated to remove the alcohol, did have a chemopreventive effect in this model [18].

Since genistein is expensive ($250/g) and available only in small amounts, only a limited number of studies in animal models have been carried out using the pure compound. In colon cancer models, genistein inhibited aberrant colonic crypt formation induced by two different carcinogens [19,20]. Genistein has also inhibited incidence, multiplicity and tumor size in the DMBA-initiated, phorbol ester-promoted skin cancer model in mice [21]. A fascinating recent study, found administration of genistein *only* during the neonatal period lowered mammary tumor incidence, multiplicity and size, and increased latency, in the DMBA-induced breast cancer model [22]. Future studies will need to address when genistein administration is most effective, doses required to obtain chemopreventive effects, and also whether there are positive synergistic effects on chemoprevention when genistein is administered in combination with daidzein or other non-isoflavone soy components.

MECHANISMS OF ACTION

At this time, many mechanisms have been put forward to account for the observed and pre-

dicted chemopreventive effects of isoflavones. Until 1987, genistein was considered a phytoestrogen, acting either as an estrogen agonist or antagonist. Since then, a variety of non-estrogenic mechanisms have been proposed. These include inhibition of protein tyrosine phosphorylation, induction of differentiation, inhibition of DNA topoisomerases, inhibition of specific cell cycle events, induction of apoptosis, inhibition of angiogenesis and antioxidant activity. The relevance of these events *in vivo* has not yet been firmly established.

Most important is whether genistein concentrations at the cancer cell level in the body reach concentrations used in cell culture experiments to demonstrate these biochemical and biological events. Daily dietary intake of genistein in heavy soy consumers is approximately 50 mg (185 μmol) [17]; in Asian diets, soy is the principal source of these isoflavones [23]. Assuming that this daily dose distributes through body water, the maximum serum genistein concentration is unlikely to exceed 3–4 μM [17]. This has been confirmed by studies in which human adults were fed 50 mg of genistein and daidzein orally [24]. The peak serum concentrations observed with this dose were 0.7 μM (0.2 μg/ml). Thus, in many experiments, genistein only shows its effects at supraphysiological, if not pharmacological, concentrations.

On a cautionary note, satisfactory analytical procedures to measure isoflavones in blood and tissues have not yet been established, and it is possible that cells are exposed to higher concentrations of genistein than currently thought. The possibility of genistein accumulation in enterohepatic circulation has not been considered. Current analytic methodologies based on gas chromatography-mass spectrometry (GC-MS) require extensive steps in the work-up procedure to separate individual conjugated forms of the isoflavones, hydrolyze the conjugates and prepare suitable volatile derivatives for GC. The variable and extensive losses in these procedures demand isotopically labeled standards suitable for reverse isotope dilution analysis. However, the deuterated isoflavones used for this purpose are themselves unstable [25], calling into question the validity of existing methods.

Reversed-phase HPLC greatly simplifies the analysis of isoflavones since the aglucones and their conjugates can be separated in one run without need for the derivatization required by GC-MS. However, sensitivity and specificity is a problem because of low blood concentrations of isoflavones [17,24]. The recent introduction of a combination of reversed-phase HPLC and electrospray ionization MS may overcome many of these difficulties [26]. However, much work needs to done in the area of initial extraction of the isoflavones from the biological matrix.

ESTROGEN AGONISM AND ANTAGONISM

Genistein and daidzein are weak estrogens; however, several authors hypothesized chemopreventive properties similar to the estrogen antagonist tamoxifen [13,27,28], which has been used extensively in the treatment of estrogen receptor-positive breast cancer and is currently being tested in a breast cancer chemoprevention trial. The possibility of a weak estrogen agonist action of genistein should be considered seriously. Zava *et al.* [29] found that genistein added in low concentrations (1–100 nM) to estrogen-free, serum-containing media stimulates growth of the human breast cancer cell line MCF-7; however, at higher concentrations (1 μM) it inhibits growth. This may have relevance to postmenopausal women with low serum concentrations of physiologic estrogens. It may also account for the lack of association between soy intake and breast cancer incidence in postmenopausal women as opposed to premenopausal women [30].

PROTEIN TYROSINE PHOSPHORYLATION

During the 1980s, there was an intense search for compounds which inhibited protein tyrosine phosphorylation of enzymes in the signal transduction cascades stimulated by some growth factors. This was relevant to cancer chemoprevention since many of the expressed proteins of the oncogenes identified at that time were either activating ligands of tyrosine kinases (*e.g.*, c-*sis*), tyrosine kinases (*erb*-B2, c-*src*), or substrates of tyrosine kinases [31]. A tyrosine kinase inhibitor therefore would have promise in chemoprevention. Genistein was "discovered" as a potent inhibitor of tyrosine phosphorylation using the epidermal growth factor receptor tyrosine kinase from A-431 cells as a screen of microbial metabolites [32]. The genistein did not come from the

microorganism but rather the soymeal used as the source of protein in the growth medium of the microorganisms that were studied. It was subsequently shown that genistein was a specific inhibitor of tyrosine, but not serine/threonine phosphorylation [33]. Now genistein is widely use as a probe to detect tyrosine kinase involvement in many cell signalling pathways, with the number of published papers doubling each year since 1989. Despite this wide use of genistein, it cannot be assumed that *in vivo* genistein acts directly as a tyrosine kinase inhibitor [34,35]. McNichol [35] presented evidence that genistein inhibits thromboxane-induced pp50 tyrosine phosphorylation in platelets by competing with the thromboxane agonist U46619 for binding to the thromboxane receptor and not on the tyrosine kinase that phosphorylates pp50.

INDUCTION OF CELL DIFFERENTIATION

In addition to inhibiting growth of leukemia cell lines, genistein also causes them to differentiate and produce hemoglobin [36,37]. However, genistein inhibits differentiation in many hemopoietic cells, although it has no effect in others [38]. Since the various stimulators of differentiation use separate signal transduction pathways, it is not known which cellular target is affected by genistein.

INHIBITION OF DNA TOPOISOMERASES

Genistein has been reported to inhibit topoisomerase activity both *in vivo* [39,40] and *in vitro* at concentrations from 20–110 μM [39–41]. The relationship between topoisomerase II inhibition and inhibition of cell growth, however, is still a matter of debate [42]. From a mechanistic standpoint, genistein inhibits topoisomerase activity by inhibiting the interaction of ATP with its binding site on topoisomerase II [43,44], stabilizing the transient DNA-topoisomerase complex observed in several experiments.

INHIBITION OF CELL CYCLE-SPECIFIC EVENTS

Genistein arrests cell growth at various stages of the cell cycle. At 20–40 μM genistein, Jurkat T leukemia cells were arrested at the G_2/M transition; whereas at 70–110 μM, they were arrested in the S-phase [45]. At the higher concentrations, tyrosine phosphorylation was also inhibited. In rat smooth muscle cells, genistein arrested in cells stimulated by PDGF at the G_0/G_1 transition; however, it had no effect on serum-induced timulation [46]. Genistein also arrested K-562 leukemia cells [36], HL-60 cells [47] and HGC-27 gastric cancer cells [48] at the G_2/M transition.

INDUCTION OF APOPTOSIS

Genistein causes apoptosis in many cell lines [49–53]. In each case the concentration of genistein (30–40 μM) was cytotoxic, and in excess of that obtained through a soy-based diet. In HL-60 cells, both genistein and the synthetic tyrosine kinase inhibitor tyrphostin AG82 caused apoptosis [49]. Since the action of genistein was only partially blocked by the tyrosine phosphatase inhibitor orthovanadate, whereas that of the tyrphostin was fully blocked, genistein must have additional or different targets from the tyrphostin. In thymocytes, genistein caused apoptosis, but unlike herbimycin, another tyrosine kinase inhibitor, did not alter tyrosine phosphorylation [53].

INHIBITION OF ANGIOGENESIS

Supply of blood and nutrients is a vital part of of primary tumor growth and the process of metastasis. Genistein, by inhibiting angiogenesis, could have an important role in preventing the appearance of cancer. Although genistein inhibits angiogenesis [54], the IC_{50} for this event is 150 μM, far in excess of observed serum concentrations in soy consumers.

ANTIOXIDANT EFFECTS

Genistein may prevent cancer as a result of its biological antioxidant properties. It has been suggested that reactive oxygen species play an important role in mutagenesis and carcinogenesis, particularly tumor promotion [55]. Genistein inhibits the production of hydrogen peroxide in response to the phorbol ester TPA in HL-60 cells, human polymorphonuclear cells, and mouse skin [56]. The effect could only be partially accounted for in terms of a chemical reaction between hydrogen peroxide and genistein, suggesting that the effect of genistein is at a

biochemical level. Indeed, genistein inhibits expression of the immediate early gene c-*fos* in the mouse skin model [21]. Genistein is particularly effective in inhibiting the production of superoxide anion stimulated by formyl-methionyl-leucyl-phenylalanine in granulocyte colony stimulating factor-primed human neutrophils with an IC_{50} close to 1 μM [57]. More research is clearly indicated in this area.

DELIVERY CHOICES FOR GENISTEIN

Since genistein is found in a food source, there is the option in chemoprevention trials to use it either as the pure compound (extracted from soy or prepared synthetically) or as a genistein-containing, soy-based foodstuff. It should be noted that the chemical form of genistein in soy varies from the aglucone in fully fermented food (miso) to a variety of glucosides in non-fermented foods [15,16]. These differences have the potential to alter the initial uptake of genistein from the intestine and its pharmacokinetics—the aglucone should be absorbed rapidly in the upper part of the small intestine, whereas the glucoside conjugates would have to undergo hydrolysis to the aglucone before absorption. The latter may also lead to bacterial metabolism of genistein prior to uptake.

The principal advantage of using a soy food as the source of genistein is that the level of genistein (25–100 mg/day) in the food does not lead to any known toxicity, having been used by the peoples of Southeast Asia for centuries. By using purified or synthetic genistein, a much larger dose can be delivered (20–40 fold larger), but this opens up the possibility of toxic side effects. Toxicity experiments in animal models and subsequent Phase I clinical trials are needed to address this issue.

The safety of using soy food products as the delivery system for genistein has already led to the initiation of clinical trials in 1994 designed to examine the effect of isolated soy protein (Supro™) on surrogate intermediate endpoint biomarkers (SIEBs) of breast cancer [58] and prostate cancer [59]. It will be important to compare the effects of the soy protein with an equivalent amount of pure genistein to determine whether other components in soy have additive or synergistic effects on SIEBs.

The other main advantage of the use of soy protein over pure genistein is the relative cost. Synthetic genistein is currently sold for $250/g, *i.e.*, $12.50 per 50 mg (the daily intake). This figure may fall to $5/g if genistein is made on a larger scale. In contrast, 50 mg of genistein is delivered in 40 g (1.5 ounces) of Supro™ (a low-fat, high quality protein) at a daily cost of $1.50. The eight-fold lower cost of genistein in soy protein has been a large factor in the design of existing clinical trials.

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REFERENCES

1. American Cancer Society. "Facts and Figures, 1994." Atlanta, GA.
2. Newell GR: Epidemiology of cancer. In DeVita VT Jr, Hellman S, Rosenberg SA (eds): "Cancer. Principles and Practice of Oncology," 2nd ed. Philadelphia: JB Lippincott, 1985, pp 151–195.
3. Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE, Mack TM: Cancer of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br J Cancer 63:963–966, 1991.
4. Dunn JE: Cancer epidemiology in populations of the United States—with emphasis on Hawaii and California—and Japan. Cancer Res 35:3240–3245, 1975.
5. Gray GE, Pike MC, Henderson BE: Breast cancer incidence and mortality rates in different countries in relation to known factors and dietary practice. Br J Cancer 39:1–7, 1979.
6. Greenwald P: Principles of cancer prevention: Diet and nutrition. In DeVita VT Jr, Hellman S, Rosenberg SA (eds): "Cancer: Principles and Practice of Oncology," 3rd ed. Philadelphia: JB Lippincott, 1989, pp 167–180.
7. Willett WC, Stampfer MJ, Colditz GA, Rosner BA,

Hennekens CH, Speizer FE: Dietary fat and the risk of breast cancer. N Engl J Med 316:22–28, 1987.
8. Willett WC, Hunter DJ, Stampfer MJ, Colditz GA, Manson JE, Spiegelman D, Rosner BA, Hennekens CH, Speizer FE: Dietary fat and fiber in relation to the risk of breast cancer. JAMA 268:2037–2044, 1992.
9. Soyatech Survey and Estimates, P.O. Box 84, Bar Harbor, ME, and Soyfoods Center Survey, P.O. Box 234, Lafayette, CA.
10. Messina M, Persky V, Setchell KDR, Barnes S: Soy intake and cancer risk: A review of *in vitro* and *in vivo* data. Nutr Cancer 21:113–131, 1994.
11. Troll W, Wiesner R, Shellabarger CJ, Holtzman S, Stone JP: Soybean diet lowers breast tumor incidence in irradiated rats. Carcinogenesis 1:469–472, 1980.
12. Barnes S, Grubbs C, Setchell KDR, Carlson J: Soybeans inhibit mammary tumors in models of breast cancer. In Pariza M (ed): "Mutagens and Carcinogens in the Diet." New York: Alan R. Liss, 1990, pp 239–253.
13. Messina M, Barnes, S: Workshop report from the Division of Cancer Etiology, National Cancer Institute, National Institutes of Health. The role of soy products in reducing risk of certain cancer. J Natl Cancer Inst 83:541–546, 1991.
14. Coward L, Barnes NC, Setchell KDR, Barnes S: The antitumor isoflavones, genistein and daidzein, in soybean foods of American and Asian diets. J Agric Food Chem 41:1961–1967, 1993.
15. Barnes S, Kirk M, Coward L: Isoflavones and their conjugates in soy foods: Extraction conditions and analysis by HPLC-mass spectrometry. J Agric Food Chem 42:2466–2474, 1994.
16. Wang H-J, Murphy PA: Isoflavone content in commercial soybean foods. J Agric Food Chem 42:1666–1673.
17. Barnes S: Effects of genistein in *in vivo* and *in vitro* models of cancer growth. J Nutr 125, 1995 (in press).
18. Barnes S, Peterson TG, Grubbs C, Setchell KDR: Potential role of dietary isoflavones in the prevention of cancer. In Jacobs MM (ed): "Diet and Cancer: Markers, Prevention and Treatment." New York: Plenum Press, 1994, pp 135–148.
19. Pereira MA, Barnes LH, Rassman VL, Kelloff GJ, Steele VE: Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. Carcinogenesis 15:1049–1054, 1994.
20. Helms JR, Gallaher JJ: The effect of dietary soy protein isolate and genistein on the development of preneoplastic lesions (aberrant crypts) in rats. J Nutr 125, 1995 (in press).
21. Wei H, Bowen R, Cai Q, Barnes S, Wang Y: Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proc Soc Exp Biol Med 208:124–130 1995.
22. Lamartiniere CA, Moore J, Holland MB, Barnes S: Neonatal genistein chemoprevents mammary carcinogenesis. Proc Soc Exp Biol Med 208:120–123, 1995.
23. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, Hasegawa T, Okada H: Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr 54:1093–1100, 1991.
24. Xu X, Wang H-J, Murphy PA, Cook L, Hendrich S: Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. J Nutr 124:825–832, 1994.
25. Adlercreutz H, Fotsis T, Bannwart C, Wahala K, Brunow G, Hase T: Isotope dilution gas chromatographic-mass spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein. Clinica Chimica Acta 199:263–278, 1991.
26. Barnes S, Coward L, Wilson L, Kirk M: Serum conjugated and unconjugated isoflavones by capillary reversed-phase HPLC-electrospray ionization-mass spectrometry. NCI Workshop on "Dietary Phytoestrogens: Cancer Cause or Cure?" Washington, DC, September, 1994.
27. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M: Non-steroidal estrogens of dietary origin: Possible roles in hormone-dependent disease. Am J Clin Nutr 40:569–578, 1984.
28. Adlercreutz H: Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest 50 (Suppl): 3–23, 1990.
29. Zava DT, Duew G: Estrogenic bioactivity of phytoestrogens in human breast cancer cells in monolayer culture. "First International Symposium on Soy in Preventing and Treating Chronic Diseases," Mesa, AZ, 1994.
30. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE: Dietary effects on breast cancer risk in Singapore. Lancet 337:1197–1200.
31. Hunter T, Cooper JA: Role of tyrosine phosphorylation in malignant transformation by viruses and in cellular growth control. Prog Nucleic Acid Res Mol Biol 29:221–232, 1983.
32. Ogawara H, Akiyama T, Ishida J, Watanabe S, Suzuki K: A specific inhibitor for tyrosine protein kinase from *Pseudomonas*. J Antibiot Tokyo 39:606–608, 1986.
33. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh NM, Shibuya M, Fukami Y: Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 262:5592–5595, 1987.
34. Peterson TG, Barnes S: Isoflavones inhibit the growth of human prostate cancer cell lines without inhibiting epidermal growth factor receptor autophosphorylation. Prostate 22:335–345, 1993.
35. McNicol A: The effects of genistein on platelet function are due to thromboxane receptor antagonism rather than inhibition of tyrosine kinase. Prostaglandins Leukot Essent Fatty Acids 48:379–384, 1993.
36. Kanatani Y, Kasukabe T, Hozumi M, Motoyoshi K, Nagata N, Honma Y: Genistein exhibits preferential cytotoxicity to a leukemogenic variant but induces

differentiation of a non-leukemogenic variant of the mouse monocytic leukemia Mm cell line. Leukemia Res 17:847–853, 1993.
37. Hunakova L, Sedlak J, Klobusicka M, Duraj J, Chorvath B: Tyrosine kinase inhibitor-induced differentiation of K-562 cells: Alterations of cell cycle and cell surface phenotype. Cancer Lett 81:81–87, 1994.
38. Bourget I, Breittmayer JP, Grenier-Brossette N, Cousin JL: CD20 monoclonal antibodies down-regulate IgM at the surface of B cells. Eur J Immunol 23: 768–771, 1993.
39. Markowits J, Linassier J, Fossé P, Couprie J, Pierre J, Jacquemin A, Saucier J-M, Le Pecq J-B, Larsen AK: Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res 49:5111–5117, 1989.
40. Constantinou A, Kiguchi K, Huberman E: Induction of differentiation and DNA strand breakage in human HL-60 and K-562 leukemia cells by genistein. Cancer Res 50:2618–2624, 1990.
41. Yamashita Y, Kawada S-Z, Nakano H: Induction of mammalian topoisomerase II dependent DNA cleavage by nonintercalative flavonoids genistein and orobol. Biochem Pharm 39:737–744, 1990.
42. Okura A, Arakawa H, Oka H, Yoshinara T, Monden Y: Effect of genistein on topoisomerase activity and the growth of [VAL 12]Ha-*ras*-transformed NIH-3T3 cells. Biochem Biophys Res Commun 157:183–189, 1988.
43. Corbett AH, Hong H, Osheroff N: Exploiting mechanistic differences between drug classes to define functional drug interaction domains on topoisomerase II. J Biol Chem 268:14394–14398, 1993.
44. Robinson M J, Corbett AH, Osheroff N: Effect of topoisomerase II-targeted drugs on enzyme-mediated DNA cleavage and ATP hydrolysis: Evidence for distinct drug interaction domains on topoisomerase II. Biochemistry 32:3638–3643, 1993.
45. Spinozzi F, Pagliacci MC, Migliorati G, Moraca R, Grignani F, Riccardi C, Nicoletti I: The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. Leukemia Res 18:431–439, 1994.
46. Kobayashi S, Nishimura J, Kanaide H: Cytosolic Ca^{2+} transients are not required for platelet-derived growth factor to induce cell cycle progression of vascular smooth muscle cells in primary culture. Actions of tyrosine kinase. J Biol Chem 269:9011–9018, 1994.
47. Traganos F, Ardelt B, Halko N, Bruno S, Darzynkiewicz Z: Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. Cancer Res 52:6200–6208, 1992.
48. Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, Nishino H, Aoike A: Genistein arrests cell cycle progression at G_2-M. Cancer Res 53: 1328–1331, 1993.
49. Bergamaschi G, Rosti V, Danova M, Ponchio L, Lucotti C, Cazzola M: Inhibitors of tyrosine phosphorylation induce apoptosis in human leukemic cell lines. Leukemia 7:2012–2018, 1993.
50. Knox KA, Gordon J: Protein tyrosine phosphorylation is mandatory for CD40-mediated rescue of germinal center B cells from apoptosis. Eur J Immunol 23:2578–2584, 1993.
51. Yanagihara K, Ito A, Toge T, Numoto M: Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. Cancer Res 53:5815–5821, 1993.
52. Carson WE, Haldar S, Baiocchi RA, Croce CM, Caligiuri MA: The c-*kit* ligand suppresses apoptosis of human natural killer cells through the upregulation of *bcl*-2. Proc Natl Acad Sci USA 91:7553–7557, 1994.
53. Azuma Y, Onishi Y, Sato Y, Kizaki H: Induction of mouse thymocyte apoptosis by inhibitors of tyrosine kinases is associated with dephosphorylation of nuclear proteins. Cell Immunol 152:271–278, 1993.
54. Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L: Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. Proc Natl Acad Sci USA 90:2690–2694, 1993.
55. Frenkel K: Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 53:127–166, 1992.
56. Wei H, Wei L, Frenkel K, Bowen R, Barnes S: Inhibition of tumor promoter induced hydrogen peroxide formation *in vitro* and *in vivo* by genistein. Nutr Cancer 20:1–12, 1993.
57. Tanimura M, Kobuchi H, Utsumi T, Yoshioka T, Kataoka S, Fujita Y, Utsumi K: Neutrophil priming by granulocyte colony stimulating factor and its modulation by protein kinase inhibitors. Biochem Pharmacol 44:1045–1052, 1992.
58. Petrakis N, Winkie J, Coward L, Kirk M, Barnes S: A clinical trial of the chemopreventive effect of a soy beverage in women at high risk for breast cancer. "First International Symposium on Soy in Preventing and Treating Chronic Diseases," Mesa, AZ, 1994.
59. Urban D, Grizzle W, Coward L, Kirk M, Barnes S: A clinical trial of the chemopreventive effect of a soy beverage in men at high risk for prostate cancer. "First International Symposium on Soy in Preventing and Treating Chronic Diseases," Mesa, AZ, 1994.