Journal of Cellular Biochemistry 104:339–356 (2008)

PROSPECT

Bioactive Food Components and Cancer Risk Reduction

Silvia D. Stan,[1,2] Siddhartha Kar,[2] Gary D. Stoner,[3] and Shivendra V. Singh[1,2]***

[1]Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
[2]University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
[3]Division of Hematology and Oncology, Department of Internal Medicine, College of Medicine and Public Health, Ohio State University, Columbus, Ohio

Abstract Research over the last three decades has provided convincing evidence to support the premise that diets rich in fruits and vegetables may be protective against the risk of different types of cancers. Initial evidence for protective effect of fruits and vegetables against cancer risk came from population-based case-control studies, which prompted intense research aimed at (a) identification of bioactive component(s) responsible for the anticancer effects of fruits and vegetables, (b) elucidation of the mechanisms by which bioactive food components may prevent cancer, and (c) determination of their efficacy for prevention of cancer in animal models. The bioactive components responsible for cancer chemopreventive effects of various edible plants have now been identified. For instance, anticancer effect of *Allium* vegetables including garlic is attributed to organosulfur compounds (e.g., diallyl trisulfide). Interestingly, unlike cancer chemotherapy drugs, many bioactive food components selectively target cancer cells. Molecular basis for selectivity of anticancer bioactive food components towards cancer cells remains elusive, but these agents appear promiscuous and target multiple signal transduction pathways to inhibit cancer cell growth in vitro and in vivo. Despite convincing observational and experimental evidence, however, limited effort has been directed towards clinical investigations to determine efficacy of bioactive food components for prevention of human cancers. This article reviews current knowledge on cancer chemopreventive effects of a few highly promising dietary constituents, including garlic-derived organosulfides, berry compounds, and cruciferous vegetable-derived isothiocyanates, and serves to illustrate complexity of the signal transduction mechanisms in cancer chemoprevention by these promising bioactive food components. J. Cell. Biochem. 104: 339–356, 2008.

Key words: bioactive food components; chemoprevention; garlic compounds; berry compounds; isothiocyanates

BACKGROUND

Accumulating evidence suggests that diet, in addition to containing cancer causing substances, also contains many cancer preventive agents [reviewed by Kelloff, 2000; Liu, 2003; Surh, 2003; Milner, 2004; Davis and Milner, 2006]. This implies that many cancers may be prevented by changes in dietary habits. It is becoming increasingly clear that many dietary agents can retard or prevent the process of carcinogenesis by multiple mechanisms including (a) enhanced detoxification of the carcinogenic intermediates through induction of

Abbreviation used: OSCs, organosulfides; ITCs, isothiocyanates; DADS, diallyl disulfide; DATS, diallyl trisulfide; SAC, S-allyl cysteine; SAMC, S-allylmercaptocysteine; Cdk1, cyclin-dependent kinase 1; ROS, reactive oxygen species; JNK, c-Jun N-terminal kinase; NMBA, *N*-nitrosomethylbenzylamine; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; PEITC, phenethyl isothiocyanate; BITC, benzyl isothiocyanate; AITC, allyl isothiocyanate; SFN, sulforaphane.

Grant sponsor: National Cancer Institute (NCI); Grant numbers: CA101753, CA113363, CA115498, CA129347, CA 096130, CA 103180; Grant sponsor: USDA; Grant numbers: 34501-13665, 38903-02313, 38903-03660.

*Correspondence to: Shivendra V. Singh, PhD, Department of Pharmacology, University of Pittsburgh Cancer Institute, Hillman Cancer Center Research Pavilion, 2.32A, 5117 Centre Avenue, Pittsburgh, PA 15213.
E-mail: singhs@upmc.edu

**Correspondence to: Gary D. Stoner, PhD, Innovation Centre, 2001 Polaris Parkway, Columbus, OH 43240.
E-mail: gary.stoner@osumc.edu

Received 1 October 2007; Accepted 4 October 2007
DOI 10.1002/jcb.21623

phase 2 drug metabolizing enzymes, (b) reduced carcinogen activation due to suppression of cytochrome P450-dependent monooxygenases, (c) selective promotion of apoptosis (cell death) in cancer cells but not in normal epithelial cells, (d) perturbations in cell cycle progression, and (e) inhibition of angiogenesis and metastasis [Kelloff, 2000; Surh, 2003; Milner, 2004; Davis and Milner, 2006]. Cancer chemopreventive effect of food constituents is also supported by epidemiological data. For example, the epidemiological correlation for protective effect of cruciferous vegetables (e.g., broccoli) and *Allium* vegetables (e.g., garlic) against cancer risk is quite strong [You et al., 1989; Verhoeven et al., 1996]. Laboratory studies have documented strong chemopreventive and possibly cancer chemotherapeutic effects of whole foods and bioactive food components against cancers of the skin, lung, breast, colon, liver, stomach, prostate and other sites [Kelloff, 2000; Liu, 2003; Surh, 2003; Milner, 2004; Davis and Milner, 2006]. The promising dietary chemopreventive compounds, which have demonstrated anticancer effects in more than one tumor model, include (−)-epigallocatechin gallate in green tea, resveratrol in grapes, lupeol in fruits like mango, delphinidin in pigmented fruits, curcumin in turmeric, sulforaphane (SFN) and other isothiocyanates (ITCs) in cruciferous vegetables, organosulfur compounds (OSCs) in *Allium* vegetables, lycopene in tomato, and genistein in soy among many others [Kelloff, 2000; Liu, 2003; Surh, 2003; Milner, 2004; Davis and Milner, 2006]. This article summarizes observational and experimental evidence supporting cancer chemopreventive potential of bioactive components from three common edible plants, including *Allium* vegetable-derived OSCs, cruciferous vegetable-derived ITCs, and berry compounds. Possible gaps in our understanding of the mechanism by which these bioactive food components may prevent cancer and the significance of such studies in relation to clinical translation of laboratory findings are also discussed.

EVIDENCE FOR ANTICANCER EFFECTS OF ORGANOSULFIDES

Epidemiological Evidence

Epidemiological studies continue to support the premise that dietary intake of *Allium* vegetables may be protective against various types of cancers, including stomach [You et al., 1989; Gao et al., 1999], colorectal [Dorant et al., 1996], esophageal [Gao et al., 1999], and prostate cancer [Hsing et al., 2002]. For instance, a population-based case-control study showed that the risk of prostate cancer was significantly lower in men consuming >10 g/day of total *Allium* vegetables than in men with total *Allium* vegetable intake of <2.2 g/day [Hsing et al., 2002].

Anticancer Effect of Organosulfides in Animal Models

The anticarcinogenic effect of *Allium* vegetables is attributed to organosulfur compounds [e.g., diallyl sulfide, diallyl disulfide (DADS), diallyl trisulfide (DATS), S-allyl cysteine (SAC), S-allylmercaptocysteine (SAMC), ajoene, etc.], which are generated upon processing (cutting or chewing) of these vegetables [Block, 1985]. *Allium* vegetable-derived OSCs are highly effective in affording protection against cancer in animal models induced by a variety of chemical carcinogens. For example, DAS has been shown to inhibit aberrant crypt foci [Wargovich et al., 1996], hepatic foci [Singh et al., 2004a], and *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumors in rats [Wargovich et al., 1988], and polycyclic aromatic hydrocarbon-induced skin carcinogenesis in mice [Singh and Shukla, 1998]. Likewise, DADS has been shown to inhibit chemically induced colon carcinogenesis in rats [Takahashi et al., 1992; Reddy et al., 1993], *N*-methyl-*N*-nitrosourea-induced rat mammary carcinogenesis [Schaffer et al., 1996], and skin tumors in mice [Dwivedi et al., 1992]. The DADS treatment also inhibited 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats [Suzui et al., 1997]. Studies from our laboratory have shown that DADS suppresses growth of H-ras oncogene-transformed tumor xenografts in nude mice without causing weight loss or any other side effects [Singh et al., 1996]. Similarly, oral gavage of 6 μmol DATS (three times per week) to male nude mice significantly inhibited growth of PC-3 human prostate cancer xenografts [Xiao et al., 2006a]. Protective effect of garlic oil against skin tumor promotion [Belman, 1983], garlic extract against methylcholanthrene-induced carcinogenesis of the uterine cervix of

mice [Hussain et al., 1990], and ajoene against skin tumor promotion in mice [Nishikawa et al., 2002] has also been documented.

Mechanisms for Anticancer Effects of Organosulfides

A review of the literature suggests that OSCs may prevent cancer by multiple mechanisms including impairment of carcinogen activation, enhanced inactivation of carcinogenic intermediates through induction of phase 2 enzymes, inhibition of post-translational modification of oncogenic Ras, induction of apoptosis, inhibition of cell cycle progression, histone modification, inhibition of angiogenesis and metastasis [reviewed by Shukla and Kalra, 2007; Herman-Antosiewicz et al., 2007a]. For example, diallyl sulfide has been reported to inhibit 2-aminofluorene-DNA adduct formation in human promyelocytic leukemia cells [Lin et al., 2002], inhibit cyclooxygenase-2 (COX-2) in HEK 293T cells [Elango et al., 2004], and competitively inhibit the activity of cytochrome P-4502E1 in a time- and NADPH-dependent manner with pseudo-first-order kinetics [Brady et al., 1991]. The diallyl sulfide, DADS and/or DATS are potent inducers of the expression of phase 2 carcinogen inactivating enzymes, including glutathione transferases in liver, lung and/or forestomach of mice [Sparnins et al., 1988; Hu et al., 1996; Singh et al., 1997, 1998].

It is also becoming clear that OSCs are promiscuous since they target multiple signal transduction pathways to trigger growth arrest and programmed cell death (apoptosis).

The ability of OSCs to cause cell cycle arrest and apoptosis induction was first documented by Milner and colleagues in human colon cancer cells [Sundaram and Milner, 1996; Knowles and Milner, 2000]. The DADS-mediated apoptosis in human colon cancer cells correlated with an increase in the levels of free intracellular calcium and the G2/M phase cell cycle arrest was accompanied by a decrease in p34 cyclin-dependent kinase (cdk1) activity, a reduction in complex formation between cdk1 and cyclin B1, and a decrease in Cdc25C protein level [Sundaram and Milner, 1996; Knowles and Milner, 2000]. Subsequently, the OSC-mediated cell cycle arrest and/or apoptosis induction was shown in human colon cancer cells [Robert et al., 2001], SH-SY5Y neuroblastoma cells [Filomeni et al., 2003], and PC-3, DU145 and LNCaP human prostate cancer cells [Xiao et al., 2004a, 2005a; Xiao and Singh, 2006; Kim et al., 2007]. More recent studies from our laboratory have offered novel insights into the mechanism by which DATS causes cell cycle arrest and apoptosis induction in human prostate cancer cells. We found that even a subtle change in OSC structure (e.g., oligosulfide chain length and the presence of terminal allyl groups) has a significant impact on its growth inhibitory and apoptosis inducing potency [Xiao et al., 2004a]. Interestingly, a normal prostate epithelial cell line is significantly more resistant to growth arrest and apoptosis induction by DATS compared with prostate cancer cells [Xiao et al., 2005a; Kim et al., 2007]. We showed for the first time that the DATS treated prostate cancer cells are not only arrested in G2 phase of the cell cycle but also in mitosis [Herman-Antosiewicz and Singh, 2005; Xiao et al., 2005a; Herman-Antosiewicz et al., 2007b]. The DATS-induced G2 phase cell cycle arrest is independent of p21 but correlates with reactive oxygen species (ROS)-dependent down-regulation and Ser216 phosphorylation of Cdc25C, a dual-specificity phosphatase partially responsible for activation of Cdk1/cyclin B kinase complex [Xiao et al., 2005a; Antosiewicz et al., 2006]. Subsequently, we reported activation of a novel checkpoint kinase1-dependent prometaphase checkpoint in DATS treated cancer cells [Herman-Antosiewicz et al., 2007b]. The DATS-mediated prometaphase arrest was associated with inhibition of anaphase promoting complex/cyclosome as revealed by accumulation of its substrates (cyclin A, cyclin B1, and securin) and hyperphosphorylation of core subunits (Cdc20 and Cdh1) of the anaphase promoting complex/cyclosome [Herman-Antosiewicz et al., 2007b]. The DATS-mediated ROS generation, which also contributed to the cell death by activating c-Jun N-terminal kinase (JNK), was caused by degradation of iron storage protein ferritin leading to elevation of labile iron pool [Antosiewicz et al., 2006; Kim et al., 2007]. The DATS-mediated G2/M phase cell cycle arrest was significantly attenuated by pretreatment with antioxidants [Xiao et al., 2005a]. A schematic of complexity of signal transduction leading to cell cycle arrest and apoptosis induction by DATS is depicted in Figure 1. Despite these advances, however, the mechanism of DATS-induced cell cycle arrest is not fully understood. For instance, the mechanism by which checkpoint

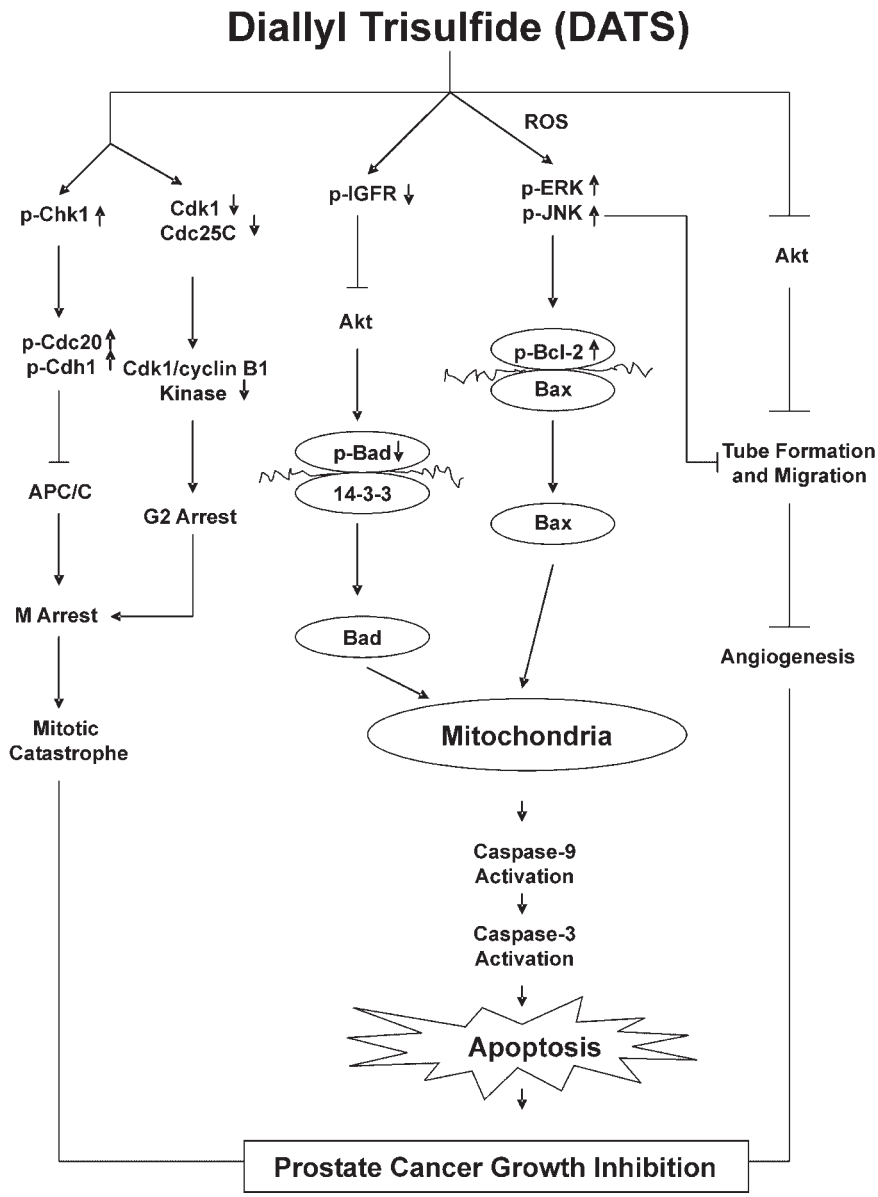

Fig. 1. Summary of signal transduction leading to cell cycle arrest, apoptosis induction, and suppression of angiogenesis by DATS. The DATS-induced G2 phase cell cycle arrest in PC-3 and DU145 human prostate cancer cells correlates with a decrease in protein levels of Cdk1 and Cdc25C leading to accumulation of inactive (Tyr15 phosphorylated) Cdk1 [Xiao et al., 2005a]. The DATS treatment causes a checkpoint kinase 1-dependent prometaphase arrest in cancer cells, which correlates with inactivation of anaphase-promoting complex/cyclosome (APC/C) as evidenced by accumulation of its substrates (cyclin A, cyclin B1, and securin) and hyperphosphorylation of APC/C subunits Cdc20 and Cdh1 [Herman-Antosiewicz and Singh, 2005; Herman-Antosiewicz et al., 2007b]. The mechanism by which DATS causes activation of checkpoint kinase 1 remains elusive but may involve ROS-dependent DNA double strand breaks. The DATS treatment causes JNK (and to some extent extracellular signal-regulated kinase)-dependent phosphorylation of Bcl-2 in PC-3/DU145 cells leading to reduced interaction between Bcl-2 and Bax and mitochondria-mediated caspase activation and apoptosis [Xiao et al., 2004a]. The DATS treatment also inactivates Akt leading to reduced phosphorylation and mitochondrial translocation of proapoptotic Bad [Xiao and Singh, 2006]. The DATS inhibits angiogenic features in human umbilical vein endothelial cells in association with inactivation of Akt and JNK activation [Xiao et al., 2006b].

kinase 1 regulates DATS-mediated inhibition of anaphase promoting complex/cyclosome and mitotic block is not fully understood. Likewise, it is unclear if the DATS-induced G2 phase cell cycle arrest is reversible and serves to allow the cell time to repair damage.

The DADS is shown to increase histone acetylation and p21(waf1/cip1) expression in

human colon tumor cell lines [Druesne et al., 2004]. In addition, Hosono et al. [2005] have documented that DATS can cause specific oxidative modification of cysteine residues Cys-12 and Cys-354 of beta-tubulin in vitro. However, it remains to be seen whether DATS causes oxidative modification of beta-tubulin cysteine in vivo.

As summarized in Figure 1, the DATS-mediated cell death in human prostate cancer cells is caspase-dependent and regulated by JNK-mediated, and to some extent extracellular signal-regulated kinase-mediated, phosphorylation of Bcl-2 (PC-3 and DU145 cells) leading to reduced interaction between Bcl-2 and Bax [Xiao et al., 2004a], inhibition of Akt kinase leading to reduced phosphorylation of proapoptotic Bcl-2 family member BAD and its translocation to the mitochondria [Xiao and Singh, 2006], and induction of proapoptotic proteins Bax and Bak [Kim et al., 2007]. The DATS-mediated suppression of PC-3 xenograft growth in vivo also correlates with induction of Bax and Bak protein expression [Xiao et al., 2006a]. A critical role for JNK in apoptosis induction by DADS has also been observed in neuroblastoma cells [Filomeni et al., 2003]. Apoptosis induction by DATS has been shown in BGC823 gastric cancer cell line [Li et al., 2006] and colon cancer cells [Hosono et al., 2005]. We have also shown previously that DATS treatment inhibits angiogenic features of human umbilical vein endothelial cells in association with suppression of vascular endothelial growth factor secretion, vascular endothelial growth factor receptor-2 down-regulation and inhibition of Akt [Xiao et al., 2006b].

Ajoene, a lipid-soluble component of garlic, induces nuclear factor-κB (NF-κB) activation, ROS generation, and apoptosis [Dirsch et al., 1998], and G2/M phase cell cycle arrest [Xu et al., 2004] in human leukemia cells. Garlic and onion oils inhibited proliferation and induced differentiation in HL-60 cells [Seki et al., 2000].

Pharmacokinetics and Human Studies With Organosulfides

A fundamental question, which remains unanswered, is whether the high μM concentrations of OSCs needed for cancer cell growth suppression and apoptosis induction in vitro are achievable in human in vivo. Limited data exist on the bioavailability of garlic compounds. The pharmacokinetics of SAC was studied in rats, mice, and dogs [Nagae et al., 1994]. The SAC was rapidly and easily absorbed in the gastrointestinal tract and distributed mainly in plasma, liver, and kidney. The bioavailability was 98.2%, 103.0%, and 87.2% in rats, mice, and dogs, respectively. The SAC was found to be mainly excreted into urine in the *N*-acetyl form in rats. On the other hand, mice excreted both SAC and the *N*-acetyl form. The half-life of SAC was longer in dogs than in rats and mice [Nagae et al., 1994]. The pharmacokinetic parameters for DATS in humans have not yet been measured, but the concentration of DATS in rat blood following treatment with 10 mg of the compound was shown to be about 31 μmol/L [Sun et al., 2006].

A preliminary, double-blind clinical trial using aged garlic extract (for 12 months at 2.4 ml/day) has recently reported a significant ($P = 0.04$) suppression of both size and number of colon adenomas in patients [Tanaka et al., 2006]. Excessive consumption of garlic can cause burning sensations, diarrhea, and allergic reactions [Siegers, 1992]. A toxicity study in mice has reported that the 50% lethal oral dose (mg/kg body weight) for allicin is 309 in males and 363 in females; for SAC is 8890 in males and 9390 in females; and for DADS is 145 in males and 130 in females [Imada, 1990]. Oral administration of 200 mg of synthetic DATS (also known as allitridum) in combination with 100 μg selenium every other day for 1 month did not cause any harmful side effects [Li et al., 2004]. Detailed toxicology of OSCs, especially DATS due to its promising preclinical effects, followed by clinical trials is needed to determine whether constituents of *Allium* vegetables may be used to prevent cancers in humans.

EVIDENCE FOR ANTICANCER EFFECTS OF BERRY CONSTITUENTS

Bioactive Components of Berries

Berries (blackberry, raspberry, strawberry, blueberry, cranberry, etc.) contain numerous bioactive compounds including polyphenols (such as several anthocyanins, ellagic acid, gallic acid, ferulic acid, protocatechuic acid, p-coumaric acid, and quercetin), phytosterols (such as β-sitosterol, stigmasterol, and kaemferol), β-carotene, α-carotene, lutein, vitamins A, C, E, folic acid, calcium, and selenium [Stoner

et al., 2006]. Black raspberries contain approximately 15–20 mg of anthocyanins and approximately 2 mg of ellagic acid per gram dry weight [Bravo, 1998; Harris et al., 2001].

Cancer Chemoprevention by Berries and Their Constituents in Animal Models

In vivo studies with ellagic acid have shown that dietary intake of this phytochemical inhibits chemically induced tumorigenesis in the lung [Boukharta et al., 1992], skin [Mukhtar et al., 1984], and esophagus [Mandal and Stoner, 1990]. In addition, dietary administration of ellagic acid has been found to inhibit the development of azoxymethane-induced small intestinal adenocarcinomas in rats [Rao et al., 1991]. Several in vivo studies with freeze-dried berries have also been reported. Berries contain compounds that influence the metabolism of NMBA leading to reduced DNA damage and reduced rate of esophageal cell proliferation compared with control animals [Stoner et al., 2006]. The inhibition of tumor development by berries was associated with down-regulation of both COX-2 and inducible nitric oxide synthase (iNOS) mRNA expression and reduction of prostaglandin E_2 (PGE_2) and nitrite levels, respectively, in the esophagus of NMBA-treated rats [Stoner et al., 2006; Chen et al., 2006a]. Dietary administration of freeze-dried black raspberries and strawberries caused inhibition of NMBA-induced esophageal tumor incidence and multiplicity in rats, and reduced the formation of O^6-methylguanine adducts in esophageal DNA [Carlton et al., 2001; Kresty et al., 2001]. Black raspberries modulated NMBA metabolism in the liver and esophagus of rats, and inhibited NMBA-induced angiogenesis in association with suppression of COX-2 and iNOS expression [Reen et al., 2006; Chen et al., 2006a,b]. Interestingly, the dietary administration of freeze-dried blueberries to NMBA-treated rats did not lead to a reduction in either the formation of O^6-methylguanine adducts in esophageal DNA or to an inhibition of esophageal tumor incidence or multiplicity [Aziz et al., 2002]. Suppression of azoxymethane-induced colon tumorigenesis in rats by administration of lyophilized black raspberries has also been reported [Harris et al., 2001]. This suppression was associated, at least in part, by a reduction in the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the urine of azoxymethane-treated rats, suggesting that the berries reduce oxidative stress in carcinogen-treated animals.

Mechanisms for Anticancer Effects of Berry Preparations and Their Constituents

Similar to *Allium* vegetable-derived OSCs, the berry compounds influence multiple signaling pathways through modulation of key regulatory transcription factors (e.g., NF-κB and AP-1), kinases (e.g., Akt), and mitogen-activated protein kinases (MAPK) leading to effects on downstream genes such as COX-2, VEGF, and iNOS [Lu et al., 2006]. Ellagic acid has been shown to cause G1 phase cell cycle arrest, inhibit cell growth, increase expression of cdk inhibitor p21, and induce apoptosis [Narayanan et al., 1999]. Ellagic acid inhibited NMBA metabolism and DNA binding in cultured rat esophagus [Mandal et al., 1988]. Ferulic acid and β-sitosterol have been shown to inhibit the growth of premalignant and malignant human oral cavity cell lines but not normal oral cavity cells [Han et al., 2005]. The anthocyanins (cyanidin glycosides) found in black raspberries were demonstrated as effective inhibitors of NF-kappa B and AP-1 expression in mouse epidermal JB-6 cells, and the ellagitannins in red raspberries inhibited the growth of human cancer cells [Hecht et al., 2006; Ross et al., 2007]. It is interesting to speculate that the inability of blueberries to inhibit NMBA-induced tumorigenesis in the rat esophagus might be due to the fact that they contain different anthocyanins than black raspberries and have very low levels of ellagitannins. These citations are discussed simply to illustrate multiplicity of signaling pathways affected by berries and their constituents and not intended to be a comprehensive review of the postulated mechanisms for their cancer chemopreventive effects.

Pharmacokinetics and Human Studies With Berry Preparations and Their Constituents

A pharmacokinetic study in healthy volunteers fed 45 g freeze-dried black raspberries for 7 days showed that the berries were tolerated well and resulted in maximum concentrations of anthocyanins and ellagic acid in plasma at 1–2 h, and in urine from 0 to 4 h [Stoner et al., 2005]. Absorption of anthocyanins and ellagic acid was less than 1% of the administered dose [Stoner et al., 2005]. Phenolic acids appeared in plasma

within 45 min following oral administration of cranberry juice [Zhang and Zuo, 2004], and anthocyanins were detected in urine 1–3 h after consumption of elderberries [Cao and Prior, 1999].

Preclinical studies in rats fed freeze-dried black raspberries or strawberries at 5% and 10% in the diet for a period of 9 months showed no evidence of histopathological changes in any organ or tissue [Stoner et al., 2007]. Clinical trials with freeze-dried berries have been recently reported. A Phase I trial to determine the safety and tolerability of 45 g per day of freeze-dried black raspberries fed for 1 week to healthy volunteers [Stoner et al., 2005], showed that the freeze-dried berries were in general well tolerated, with four of the 11 subjects presenting mild to moderate constipation. Phase II clinical trials with freeze-dried black raspberries are underway in patients at high risk of oral (i.e., patients with oral dysplasia), esophageal (i.e., patients with Barrett's esophagus or esophageal dysplasia) and colon cancers, to determine whether berries will influence the progression of these diseases [Stoner et al., 2007]. Topical application of a 10% black raspberry gel to the surface of oral dysplastic lesions in 17 patients for a period of 6 weeks led to histologic regression in a subset of patients, as well as a significant reduction in loss of heterozygosity (LOH) at three tumor suppressor gene loci [Shumway et al., 2007]. Oral administration of 32 or 45 g/day (female and male, respectively) of freeze-dried black raspberries for 6 months to 10 patients with Barrett's esophagus did not result in a reduction in the length of the Barrett's lesion however, it led to reductions in the urinary excretion of two markers of oxidative stress, 8-epi-prostaglandin F2α (8-Iso-PGF2) and 8-OHdG [Kresty et al., 2006]. A clinical trial to evaluate the effect of freeze-dried strawberries on the progression of esophageal dysplasia to squamous cell carcinoma is underway in China, and trials are ongoing in the U.S. in both colon cancer patients and in patients with familial adenomatous polyposis (FAP) to determine if freeze-dried black raspberries will modulate the expression of colon cancer genes and/or regress rectal polyps in patients with FAP, respectively [Stoner et al., 2007]. We eagerly await results from these studies to gauge into potential application of berries for cancer chemoprevention. Only a few berry-derived compounds have been evaluated for chemoprevention efficacy in human clinical trials. A recent clinical trial evaluated the effect of a combination treatment of 480 mg curcumin and 20 mg quercetin (administered orally, three times a day) for adenomas in five familial adenomatous patients [Cruz-Correa et al., 2006]. The combination treatment was found to reduce the number and size of ileal and rectal adenomas in patients without appreciable toxicity [Cruz-Correa et al., 2006].

EVIDENCE FOR ANTICANCER EFFECTS OF ISOTHIOCYANATES

Epidemiological Data

Several case-control and prospective cohort studies have found that high intake of cruciferous vegetables is associated with a lower risk for lung cancer [Verhoeven et al., 1996; London et al., 2000; Higdon et al., 2007], while other prospective studies of US men [Feskanich et al., 2000] and European men and women [Miller et al., 2004] found no such inverse correlation. A number of case-control studies and a prospective study in Dutch adults [Verhoeven et al., 1996; Voorrips et al., 2000] found that high intake of cruciferous vegetables was associated with reduced colorectal cancer risk, while another prospective study did not reveal such an inverse association [Michels et al., 2000]. The inverse association between consumption of brassica vegetables and cancer risk has also been shown for prostate, endometrial, ovarian, and breast cancers [Verhoeven et al., 1996; Fowke et al., 2003; Ambrosone et al., 2004]. Recent studies have also suggested that the preventive effects of cruciferous vegetables on cancer risk might be influenced by the genetic polymorphisms including polymorphisms at glutathione transferase gene loci [Lampe and Peterson, 2002; Seow et al., 2002].

Cancer Chemoprevention by ITCs in Animal Models

The cancer chemopreventive effect of cruciferous vegetables is attributed to ITCs, which occur naturally as thioglucoside conjugates (glucosinolates) and distinguish them from other vegetables [Fahey et al., 2001]. The ITCs are hydrolysis products of glucosinolates and generated through catalytic mediation of myrosinase, which is released upon processing

(cutting or chewing) of cruciferous vegetables from a compartment separated from glucosinolates [Fahey et al., 2001]. Evidence exists for conversion of glucosinolates to ITCs by the gut microflora [Fahey et al., 2001]. ITCs have a common basic skeleton but differ in their terminal R-group, which can be an alkyl, alkenyl, alkylthioalkyl, aryl, beta-hydroxyalkyl or indolylmethyl group. At least 120 different glucosinolates have been identified [Fahey et al., 2001]. The widely studied ITCs include phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), SFN, and allyl isothiocyanate (AITC).

The ITCs have been reported as effective chemopreventive agents in several animal models. For instance, PEITC and phenyl propyl-ITC have been shown to reduce tumor incidence in the esophagus of NMBA-treated rats [Stoner et al., 1991; Wilkinson et al., 1995]. Protective effect of PEITC has also been reported against lung tumorigenesis induced by the tobacco-derived carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mice [Morse et al., 1989, 1991; Hecht et al., 2000] and rats [Chung et al., 1996], and lung and pancreatic tumors induced by *N*-nitrosobis(2-oxopropyl)-amine in hamster [Nishikawa et al., 1996]. On the other hand, PEITC administration was ineffective in prevention of lung tumorigenesis induced by benzo[a]pyrene in mice [Adam-Rodwell et al., 1993]. To our surprise, treatment with dimethylbenz[a]anthracene plus PEITC increased the multiplicity of mammary tumors relative to that observed with carcinogen treatment alone in rats [Lubet et al., 1997]. Inhibitory effects of BITC have been shown in models of mouse lung and forestomach tumorigenesis induced by benzo[a]pyrene [Wattenberg, 1987]. However, BITC had no effect on inhibition of lung tumors induced by diethylnitrosamine in mice [Wattenberg, 1987] or esophageal tumors induced by NMBA in rats [Wilkinson et al., 1995]. These results indicated that even a subtle difference in ITC structure (e.g., the alkyl chain length between PEITC and BITC) could have a significant impact on its ability to prevent chemically induced cancers in vivo. However, the mechanism behind differential response of PEITC and BITC for prevention of chemically induced cancers is not fully understood and requires further investigation. In addition to prevention of chemically induced cancers, PEITC has been shown to inhibit growth of prostate cancer xenografts in nude mice [Xiao et al., 2005b, 2006c].

The SFN is another widely studied ITC compound that has generated a great deal of research interest due to its interesting biological effects. For example, SFN has been shown to inhibit mammary tumorigenesis induced by dimethylbenz(a)anthracene in rats [Zhang et al., 1994], formation of aberrant crypt foci induced by azoxymethane in rats [Chung et al., 2000], forestomach tumorigenesis induced by benzo[a]pyrene in mice [Fahey et al., 2002], and 7,12-dimethylbenz(α)anthracene/12-*O*-tetradecanoylphorbol 13-acetate-induced skin tumorigenesis in mice [Gills et al., 2006]. We have shown previously that orally administrated SFN significantly retards growth of PC-3 human prostate cancer xenografts in athymic mice without causing weight loss or any other side effects [Singh et al., 2004b]. Feeding of Apc/Min/+ mice with diets supplemented with 300 and 600 parts per million SFN for 3 weeks resulted in statistically significant inhibition of polyp size and number [Hu et al., 2006].

Mechanisms for Anticancer Effects of ITCs

The mechanism for cancer chemoprevention by ITCs possibly involves both inhibition of cytochrome P450s-dependent carcinogen activation and induction of phase 2 enzymes responsible for detoxification of carcinogenic intermediates [reviewed by Hecht, 1999; Conaway et al., 2002; Fimognari and Hrelia, 2007]. For example, SFN has been shown to cause potent induction of phase 2 enzymes in human prostate cancer cells [Brooks et al., 2001]. Interestingly, Affymetrix whole genome microarray analyses using gastric mucosal tissues collected from volunteers 6 h after administration of high glucosinolate broccoli (a rich source of SFN) or water failed to detect any changes in expression of genes associated with xenobiotic metabolism and cell cycle control [Gasper et al., 2007]. The effect of SFN administration on the immune system was studied recently using BALB/c mice [Thejass and Kuttan, 2006]. Intraperitoneal administration of five doses of SFN (500 μg/dose/animal/day) was found to increase total white blood cell count, bone marrow cellularity, and α-esterase positive cells [Thejass and Kuttan, 2006]. Treatment with SFN together

with sheep red blood cells as an antigen caused an increase in the circulating antibody titer [Thejass and Kuttan, 2006]. The SFN was shown to suppress angiogenesis and disrupt endothelial mitotic progression and microtubule polymerization [Jackson et al., 2007]. We have shown recently that PEITC inhibits angiogenesis in vitro and ex vivo [Xiao and Singh, 2007] at pharmacologically achievable concentrations. The PEITC-mediated inhibition of angiogenesis correlated with suppression of vascular endothelial growth factor secretion and inactivation of Akt [Xiao and Singh, 2007]. We speculate that inhibition of angiogenesis may be an important mechanism in cancer chemoprevention by ITCs. Both AITC and PEITC inhibited tumor-specific angiogenesis by down-regulating nitric oxide and tumor necrosis factor-alpha production [Thejass and Kuttan, 2007]. The SFN was shown to inhibit histone deacetylase [Myzak et al., 2004, 2006]. Suppression of NF-κB and NF-κB-regulated gene expression (vascular endothelial growth factor, Bcl-xL, cyclin D1) by SFN and PEITC has also been documented [Xu et al., 2005].

Several ITCs, including PEITC, BITC, SFN, and AITC, have been shown to inhibit cell growth and cell cycle progression and cause apoptosis in various cell types. For example, SFN was reported to inhibit cell proliferation, induce cell cycle arrest and apoptosis in human colon [Gamet-Payrastre et al., 2000], leukemia [Fimognari et al., 2002], and prostate cancer cells [Singh et al., 2004a,c, 2005; Choi and Singh, 2005; Choi et al., 2007]. Recent studies from our laboratory have demonstrated that SFN treatment causes irreversible G2/M phase cell cycle arrest in human prostate cancer cells that is associated with activation of checkpoint kinase 2, which promotes Ser216 phosphorylation of Cdc25C leading to its translocation from nucleus to the cytosol [Singh et al., 2004c]. A diagram showing mechanism of SFN-induced G2/M phase cell cycle arrest in human prostate cancer cells, based on the results from our laboratory, is presented in Figure 2. However, it is unclear if the checkpoint kinase-2/Cdc25C-mediated G2/M phase cell cycle arrest is unique to the human prostate cancer cells or to SFN. Interestingly, Cho et al. [2005] showed that the G2/M phase cell cycle arrest in DU145 human prostate cancer cell line is accompanied by JNK activation and pharmacological inhibition of JNK activation confers significant protection against the cell cycle arrest. The G2/M phase cell cycle arrest by SFN treatment has also been observed in other cellular systems including HT29 human colon cancer cell line [Gamet-Payrastre et al., 2000], F3II mouse sarcomatoid mammary carcinoma cell line [Jackson and Singletary, 2004], and human benign prostate hyperplasia epithelial cell line BPH-1 [Myzak et al., 2006]. In contrast, previous studies by Chiao et al. [2002] have documented a G0/G1 phase cell cycle arrest in LNCaP cells following a 24 h exposure to 3 and 9 μM SFN. In a follow-up study, the same group of investigators reported enrichment of G1 fraction in DU145 human prostate cancer cells under similar conditions of SFN treatment [Wang et al., 2004], which is in sharp contrast to the G2/M phase arrest observed by Cho et al. [2005] in the same cell line (10 μM SFN for 24 h). The G0/G1 phase cell cycle arrest in SFN treated LNCaP cells was also not observed in another study [Herman-Antosiewicz et al., 2007c]. Discrepancy in cell cycle responses to SFN has also been reported in HT29 human colon cancer cells [Gamet-Payrastre et al., 2000; Shen et al., 2006]. The reasons for discrepancy in cell cycle effects of SFN remain to be explained. The G2/M phase cell cycle arrest in cancer cells in response to treatment with other ITCs has also been observed, including PEITC [Zhang et al., 2003; Visanji et al., 2004; Xiao et al., 2004b], BITC [Zhang et al., 2003; Miyoshi et al., 2004; Srivastava and Singh, 2004; Visanji et al., 2004; Xiao et al., 2006d], and AITC [Xiao et al., 2003]. A previous study showed that BITC-mediated cell cycle arrest in leukemia cells correlated with activation of p38 and pharmacological inhibition of this MAPK attenuated the cell cycle arrest [Miyoshi et al., 2004]. However, further studies are needed to determine whether involvement of p38 in ITC-mediated cell cycle arrest is restricted to leukemia cells or BITC.

Significant progress has been made in our understanding of the mechanism by which ITCs cause cell death. First, similar to OSCs, ITCs seem selective towards cancer cells since normal epithelial cells display significant resistance towards ITC-induced apoptosis [Choi and Singh, 2005; Xiao et al., 2005b, 2006d; Trachootham et al., 2006]. The second common theme emerging from molecular analysis of ITCs is that they produce ROS to initiate apoptotic signal transduction in various cell

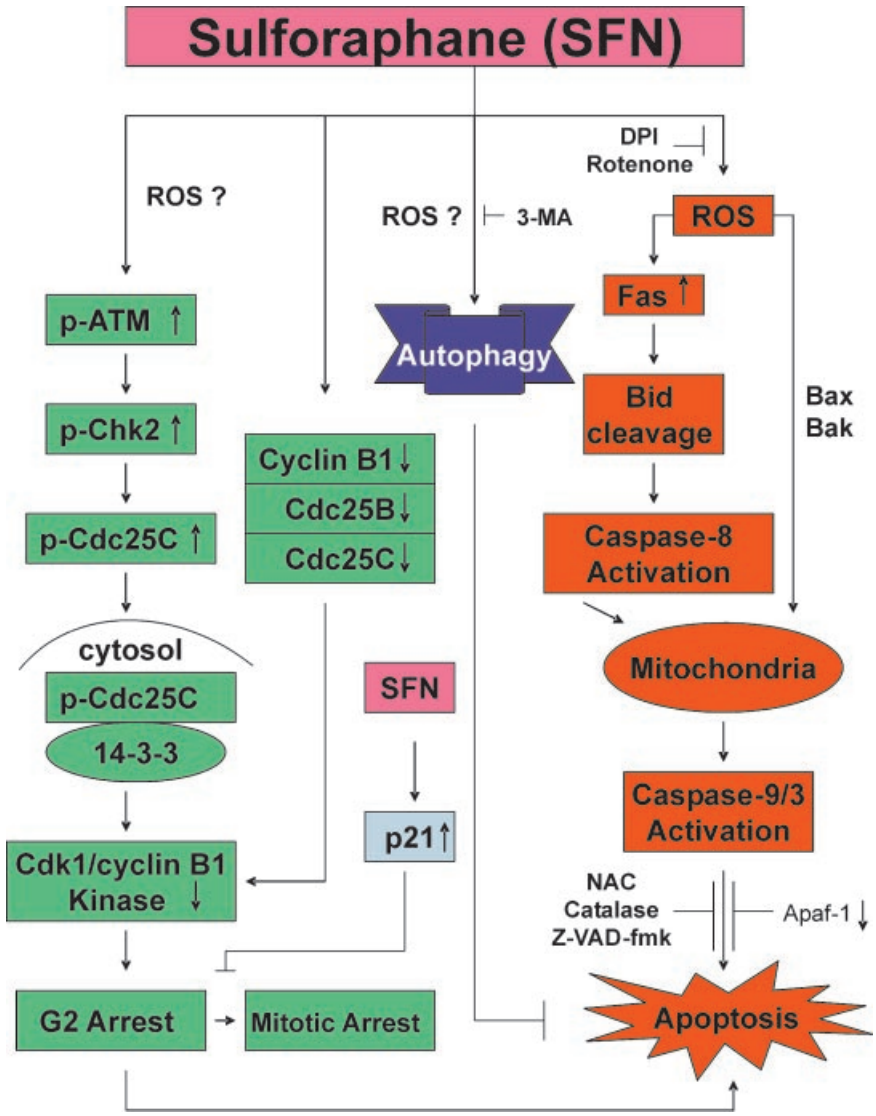

Fig. 2. Proposed mechanisms for SFN-induced cell cycle arrest and apoptosis induction in human prostate cancer cells. The SFN treatment causes activation of checkpoint kinase-2 leading to phosphorylation of Cdc25C at Ser 216 and its sequestration in the cytosol due to increased binding with 14-3-3 in PC-3 cells. The SFN treatment also decreases protein levels of cyclin B1, Cdc25B, and Cdc25C. The net result of these effects is inhibition of Cdk1/cyclin B kinase complex and G2/M progression [Singh et al., 2004c]. The SFN treated LNCaP human prostate cancer cells are also arrested in mitosis which correlates with p21 induction. The SFN-mediated p21 induction serves to protect against SFN-mediated mitotic arrest [Herman-Antosiewicz et al., 2007c]. The SFN-induced apoptosis in PC-3/DU145 cells correlates with ROS generation and activation of extrinsic caspase cascade (due to Fas induction leading to caspase-8 activation) and intrinsic caspase cascade (Bax and Bak regulated activation of caspase-9) [Singh et al., 2004a, 2005]. The SFN-induced apoptosis is inhibited by overexpression of Bcl-xL but not Bcl-2 in PC-3 cells [Singh et al., 2005], and SV40 immortalized mouse embryonic fibroblasts derived from Bax, Bak, and Bid knockout mice are significantly more resistant to apoptosis induction by SFN compared with wild-type fibroblasts [Choi and Singh, 2005; Singh et al., 2005]. The SFN-induced apoptosis in LNCaP cells correlates with stabilization of p53, activation of Bax, down-regulation of inhibitor of apoptosis family proteins, and induction of Apaf-1 [Choi et al., 2007]. The Apaf-1, but not p53, protein knockdown confers significant protection against SFN-induced apoptosis in LNCaP cells [Choi et al., 2007]. The SFN treatment also causes autophagy in PC-3/LNCaP cells characterized by appearance of membranous vacuoles in the cytoplasm as revealed by transmission electron microscopy and formation of acidic vesicular organelles as revealed by fluorescence microscopy following staining with the lysosomotropic agent acridine orange [Herman-Antosiewicz et al., 2006]. The SFN-induced autophagy correlates with up-regulation, processing, and recruitment to autophagosomes of microtubule-associated protein 1 light chain 3 and attenuated in the presence of a specific inhibitor of autophagy (3-methyladenine; 3-MA). The SFN-induced apoptosis is enhanced in the presence of 3-MA indicating that autophagy represents a defense mechanism against SFN-induced apoptosis [Herman-Antosiewicz et al., 2006].

types, including human prostate and breast cancer cells, *H-Ras*^{V12} transformed ovarian epithelial cells, and *Bcr-Abl* overexpressing hematopoietic cells [Cho et al., 2005; Singh et al., 2005; Trachootham et al., 2006; Xiao et al., 2006d]. The ITC-mediated apoptosis is significantly attenuated by antioxidants and overexpression of catalase [Singh et al., 2005; Trachootham et al., 2006; Xiao et al., 2006c,d]. However, the mechanism by which ITCs cause ROS generation is not fully understood, although a role for mitochondria in this process has been suggested by some studies [Nakamura et al., 2002; Singh et al., 2005]. Third, the ITC-mediated apoptosis is caspase-dependent and appears to involve both intrinsic and extrinsic caspase cascades [Singh et al., 2005; Tang and Zhang, 2005; Xiao et al., 2005b, 2006d]. Fourth, the ITC-mediated apoptosis correlates with changes in Bcl-2 family protein levels and Bax activation, and deficiency of Bax and Bak, but not Bid, confers significant protection against ITC-mediated cell death [Choi and Singh, 2005; Singh et al., 2005; Xiao et al., 2005b, 2006d]. Involvement of inhibitor of apoptosis family proteins and Apaf-1 in regulation of SFN-induced apoptosis was suggested in prostate cancer cells [Choi et al., 2007]. More recent studies from our laboratory have documented certain novel responses to ITCs. For instance, we are the first to show that SFN treatment causes autophagy in PC-3 and LNCaP human prostate cancer cells, which serves to protect against apoptosis induction by sequestering mitochondria in autophagosome-like structures (revealed by transmission electron microscopy) and delaying release of apoptogenic molecules from mitochondria to the cytosol [Herman-Antosiewicz et al., 2006]. The mechanism by which SFN causes autophagy remains elusive. Similarly, it is unclear if autophagic protection against cell death is unique to SFN or applies to other ITCs; although treatment of MDA-MB-231 human breast cancer cells with BITC results in formation of autophagic vacuoles [Xiao et al., 2006d]. We are also the first to report that PEITC inhibits cap-dependent translation by regulating the level and phosphorylation of 4E-BP1 [Hu et al., 2007]. The SFN was shown to sensitize human hepatoma cells to apoptosis induction by tumor necrosis factor-related apoptosis inducing-ligand through ROS-mediated up-regulation of death-receptor 5 [Kim et al., 2006].

Pharmacokinetics and Human Studies With ITCs

A pharmacokinetics study of PEITC in rats showed that ^{14}C-PEITC was readily absorbed and distributed to all major tissues with a t_{max} (time to reach maximal plasma concentration) of 2.9 h and an elimination half-life of 21.7 h after oral administration of 50 μmol/kg body weight [Conaway et al., 1999]. In another pharmacokinetic study in rats, PEITC was shown to be stable in biological samples with increased stability under refrigerated conditions [Ji et al., 2005]. The PEITC had high oral bioavailability, low clearance, and high protein binding in rats and nonlinear elimination and distribution occurred following the administration of high doses of PEITC [Ji et al., 2005]. The maximal plasma concentration of PEITC (C_{max}) following ingestion of 100 g watercress ranged between 673 and 1155 nM (mean: 928 ± 250 nM) with t_{max} of about 2.1 ± 1.1 h [Liebes et al., 2001]. A C_{max} between 0.64 and 1.4 μM (mean: 1.04 ± 0.22 μM) of total ITC in three subjects taking a single dose PEITC (40 mg) was reported in another study [Ji and Morris, 2003]. The pharmacokinetic parameters for SFN have also been studied. The SFN was detectable in the plasma of rats orally dosed with 50 μmol SFN after 1 h, peaked around 20 μM after 4 h and declined with a half life of about 2.2 h [Hu et al., 2004]. Previous studies have also shown that SFN can accumulate in cells in up to mM concentrations [Ye and Zhang, 2001].

PEITC has entered human clinical trials. A Phase I clinical trial for the prevention of lung cancer in smokers is currently ongoing at NCI (NYU-9905, NCI-P00-0151). Eight healthy women undergoing reduction mammoplasty were given a broccoli sprout preparation containing 200 μmol of SFN 1 h prior to surgery [Cornblatt et al., 2007]. The SFN metabolites were readily measurable in the mammary gland [Cornblatt et al., 2007], providing a strong rationale for evaluating the protective effects of SFN-rich broccoli sprout preparations in future clinical trials for breast cancer.

CONCLUDING REMARKS AND FUTURE DIRECTION

Epidemiological, experimental, and clinical evidence accumulated in the last few decades

suggest that fruit and vegetable-derived compounds may have beneficial effects in preventing several types of cancer. With their relatively low or no toxicity, food-derived bioactive components continue to remain highly promising cancer chemopreventive agents. Characterized by multiple mechanisms of action, the anticarcinogenic effects of bioactive food components have been shown both in vitro and in vivo. Bioactive food components can act as cytochrome P450 inhibitors, phase 2 inducers, inducers of cell cycle arrest and apoptosis, and inhibitors of angiogenesis. Even though much has been learned about the molecular pathways affected by bioactive food components, significant gaps still exist in our understanding of the mechanisms by which these compounds may prevent cancer. For instance, as discussed above, the precise mechanism of checkpoint kinase 1-dependent inactivation of anaphase promoting complex/cyclosome in DATS-mediated prometaphase arrest is not known. Likewise, the mechanism by which ITCs cause ROS generation to trigger complex signal transduction still remains elusive. Thus further studies are clearly needed to define the mechanisms by which dietary bioactive components affect cellular machinery. For instance, the intrinsic value of defining the mechanism by which bioactive food components cause cell cycle arrest and apoptosis induction may be appreciated in many ways including (a) identification and in vivo validation of biomarker(s) of their efficacy potentially useful in future clinical trials, (b) rational design of second generation analogues with improved chemopreventive index (higher efficacy and reduced toxicity, if any), (c) optimization of chemopreventive regimens to avoid potential adverse interactions with other agents/drugs, and (d) rational design of synergistic combinations using mechanistically distinct agent(s) to achieve greater chemopreventive efficacy. Biomarker development must proceed in a systematic manner beginning with identification of molecular target(s) and validation of its/their functional significance using well characterized cellular systems followed by in vivo validation of the cellular findings in appropriate animal models. Moreover, previous studies have relied on correlative observations (e.g., change in protein levels or gene expression) to draw significant conclusions concerning mechanism of action of these compounds. As an example, previous studies including our own have revealed that BITC-induced apoptosis correlates with changes in levels of Bcl-2, Bcl-xL and Bak yet no attempt has been made to experimentally verify the contribution of these proteins to the cell death caused by BITC, which underscores the significance of more in-depth molecular studies to determine the mechanism of their action.

It is interesting to note that many dietary cancer chemopreventive agents (e.g., DATS and SFN) cause ROS generation to initiate signaling events leading to cell cycle arrest and apoptosis induction in cancer cells [reviewed by Fimognari and Hrelia, 2007; Herman-Antosiewicz et al., 2007a]. Because ROS generation is implicated in pathogenesis of many chronic diseases including cancer, the potential side effects of ROS production by bioactive food components cannot be ignored. However, we are tempted to speculate that ROS generation by cancer chemopreventive food components may not be harmful because: (a) these agents are abundant in many edible plants consumed by humans on a daily basis yet epidemiological studies continue to support the premise that dietary intake of these vegetables may reduce the risk of different types of malignancies, (b) majority of the cancer chemopreventive bioactive food components are nontoxic in animals, and (c) normal epithelial cells are resistant to growth arrest by several cancer chemopreventive bioactive food constituents compared with cancer cells. It is possible that ROS generation by bioactive food constituents in cancer cells is transient and serves to trigger the apoptosis signaling cascade. However, further studies are needed to systematically explore these possibilities.

More clinical trials are needed to determine whether these promising bioactive food components can prevent cancer in humans. Also, more studies are required to understand the bioavailability and pharmacokinetics of some of these compounds. A future better approach might also involve chemoprevention using combinations rather than individual agents.

ACKNOWLEDGMENTS

The research cited in this review article from the authors laboratories was supported by the National Cancer Institute (NCI) grants CA101753, CA113363, CA115498, and CA129347 (to S.V.S.), and NCI grants CA 096130, CA

103180 and USDA grants 34501-13665, 38903-02313, and 38903-03660 (to G.D.S.).

REFERENCES

Adam-Rodwell G, Morse MA, Stoner GD. 1993. The effects of phenethyl isothiocyanate on benzo[a]pyrene-induced tumors and DNA adducts in A/J mouse lung. Cancer Lett 71:35–42.

Ambrosone CB, McCann SE, Freudenheim JL, Marshall JR, Zhang Y, Shields PG. 2004. Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. J Nutr 134:1134–1138.

Antosiewicz J, Herman-Antosiewicz A, Marynowski SW, Singh SV. 2006. c-Jun NH_2-terminal kinase signaling axis regulates diallyl trisulfide-induced generation of reactive oxygen species and cell cycle arrest in human prostate cancer cells. Cancer Res 66:5379–5386.

Aziz RM, Nines R, Rodrigo K, Harris K, Hudson T, Gupta A, Morse M, Carlton P, Stoner GD. 2002. The effect of freeze-dried blueberries on N-nitrosomethylbenzylamine tumorigenesis in the rat esophagus. Pharm Biol 40:43–49.

Belman S. 1983. Onion and garlic oils inhibit tumor promotion. Carcinogenesis 4:1063–1065.

Block E. 1985. The chemistry of garlic and onions. Sci Am 252:114–119.

Boukharta M, Jalbert G, Castonguay A. 1992. Biodistribution of ellagic acid and dose-related inhibition of lung tumorigenesis in A/J mice. Nutr Cancer 18:181–189.

Brady JF, Ishizaki H, Fukuto JM, Lin MC, Fadel A, Gapac JM, Yang CS. 1991. Inhibition of cytochrome P-450 2E1 by diallyl sulfide and its metabolites. Chem Res Toxicol 4:642–647.

Bravo L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333.

Brooks JD, Paton VG, Vidanes G. 2001. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol Biomarkers Prev 10:949–954.

Cao G, Prior RL. 1999. Anthocyanins are detected in human plasma after oral administration of an elderberry extract. Clin Chem 45:574–576.

Carlton PS, Kresty LA, Siglin JC, Morse MA, Lu J, Morgan C, Stoner GD. 2001. Inhibition of *N*-nitrosomethylbenzylamine-induced tumorigenesis in the rat esophagus by dietary freeze-dried strawberries. Carcinogenesis 22:441–446.

Chen T, Hwang H, Rose ME, Nines RG, Stoner GD. 2006a. Chemopreventive properties of black raspberries in *N*-nitrosomethylbenzylamine-induced rat esophageal tumorigenesis: Down-regulation of cyclooxygenase-2, inducible nitric oxide synthase, and c-Jun. Cancer Res 66:2853–2859.

Chen T, Rose ME, Hwang H, Nines RG, Stoner GD. 2006b. Black raspberries inhibit *N*-nitrosomethylbenzylamine (NMBA)-induced angiogenesis in rat esophagus parallel to the suppression of COX-2 and iNOS. Carcinogenesis 27:2301–2307.

Chiao JW, Chung FL, Ahmed T, Mittelman A, Conaway CC. 2002. Sulforaphane and its metabolites mediate growth arrest and apoptosis in human prostate cancer cells. Int J Oncol 20:631–635.

Cho SD, Li G, Hu H, Jiang C, Kang KS, Lee YS, Kim SH, Lu J. 2005. Involvement of c-Jun N-terminal kinase in G2/M arrest and caspase-mediated apoptosis induced by sulforaphane in DU145 prostate cancer cells. Nutr Cancer 52:213–224.

Choi S, Singh SV. 2005. Bax and Bak are required for apoptosis induction by sulforaphane, a cruciferous vegetable derived cancer chemopreventive agent. Cancer Res 65:2035–2043.

Choi S, Lew KL, Xiao H, Herman-Antosiewicz A, Xiao D, Brown CK, Singh SV. 2007. D,L-Sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. Carcinogenesis 28:151–162.

Chung FL, Kelloff G, Steele V, Pittman B, Zang E, Jiao D, Rigotty J, Choi CI, Rivenson A. 1996. Chemopreventive efficacy of arylalkyl isothiocyanates and *N*-acetylcysteine for lung tumorigenesis in Fischer rats. Cancer Res 56:772–778.

Chung FL, Conaway CC, Rao CV, Reddy BS. 2000. Chemoprevention of colonic aberrant crypt foci in Fisher rats by sulforaphane and phenethyl isothiocyanate. Carcinogenesis 21:2287–2291.

Conaway CC, Jiao D, Kohri T, Liebes L, Chung FL. 1999. Disposition and pharmacokinetics of phenethyl isothiocyanate and 6-phenylhexyl isothiocyanate in F344 rats. Drug Metab Dispos 27:13–20.

Conaway CC, Yang YM, Chung FL. 2002. Isothiocyanates as cancer chemopreventive agents: Their biological activities and metabolism in rodents and humans. Curr Drug Metab 3:233–255.

Cornblatt BS, Ye L, Dinkova-Kostova AT, Erb M, Fahey JW, Singh NK, Chen MS, Stierer T, Garrett-Mayer E, Argani P, Davidson NE, Talalay P, Kensler TW, Visvanathan K. 2007. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. Carcinogenesis 28:1485–1490.

Cruz-Correa M, Shoskes DA, Sanchez P, Zhao R, Hylind LM, Wexner SD, Giardiello FM. 2006. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. Clin Gastroenterol Hepatol 4:1035–1038.

Davis CD, Milner JA. 2006. Diet and cancer prevention. In: Temple NJ, Wilson T, Jacobs DR, editors. Nutritional health: Strategies for disease prevention. Totowa, NJ: Humana Press. pp 151–171.

Dirsch VM, Gerbes AL, Vollmar AM. 1998. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. Mol Pharmacol 53:402–407.

Dorant E, Van den Brandt PA, Goldbohm RA. 1996. A prospective cohort study on the relationship between onion and leek consumption, garlic supplement use and the risk of colorectal carcinoma in The Netherlands. Carcinogenesis 17:477–484.

Druesne N, Pagniez A, Mayeur C, Thomas M, Cherbuy C, Duee PH, Martel P, Chaumontet C. 2004. Diallyl disulfide (DADS) increases histone acetylation and p21(waf1/cip1) expression in human colon tumor cell lines. Carcinogenesis 25:1227–1236.

Dwivedi C, Rohlfs S, Jarvis D, Engineer FN. 1992. Chemoprevention of chemically induced skin tumor development by diallyl sulfide and diallyl disulfide. Pharm Res 9:1668–1670.

Elango EM, Asita H, Nidhi G, Seema P, Banerji A, Kuriakose MA. 2004. Inhibition of cyclooxygenase-2 by diallyl sulfides (DAS) in HEK 293T cells. J Appl Genet 45: 469–471.

Fahey JW, Zalcmann AT, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51.

Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A. 2002. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci 91:3147–3150.

Feskanich D, Ziegler RG, Michaud DS, Giovannucci EL, Speizer FE, Willett WC, Colditz GA. 2000. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. J Natl Cancer Inst 92: 1812–1823.

Filomeni G, Aquilano K, Rotilio G, Ciriolo MR. 2003. Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. Cancer Res 63: 5940–5949.

Fimognari C, Hrelia P. 2007. Sulforaphane as a promising molecule for fighting cancer. Mutat Res 635:90–104.

Fimognari C, Nüsse M, Cesari R, Iori R, Cantelli-Forti G, Hrelia P. 2002. Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. Carcinogenesis 23:581–586.

Fowke JH, Chung FL, Jin F, Qi D, Cai Q, Conaway C, Cheng JR, Shu XO, Gao YT, Zheng W. 2003. Urinary isothiocyanate levels, brassica, and human breast cancer. Cancer Res 63:3980–3986.

Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, Chevolleau S, Gasc N, Tulliez J, Terce F. 2000. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer Res 60:1426–1433.

Gao CM, Takezaki JH, Ding JH, Li MS, Tajima K. 1999. Protective effect of *Allium* vegetables against both esophageal and stomach cancer: A simultaneous case-referent study of a high-epidemic area in Jiangsu Province, China. Jpn J Cancer Res 90:614–621.

Gasper AV, Traka M, Bacon JR, Smith JA, Taylor MA, Hawkey CJ, Barrett DA, Mithen RF. 2007. Consuming broccoli does not induce genes associated with xenobiotic metabolism and cell cycle control in human gastric mucosa. Nutr Cancer 137:1718–1724.

Gills JJ, Jeffery EH, Matusheski NV, Moon RC, Lantvit DD, Pezzuto JM. 2006. Sulforaphane prevents mouse skin tumorigenesis during the stage of promotion. Cancer Lett 236:72–79.

Han C, Ding H, Casto B, Stoner GD, D'Ambrosio S. 2005. Inhibition of the growth of premalignant and malignant human oral cell lines by extracts and components of black raspberries. Nutr Cancer 51:207–217.

Harris GK, Gupta A, Nines RG, Kresty LA, Habib SG, Frankel WL, LaPerle K, Gallaher DD, Schwartz SJ, Stoner GD. 2001. Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2′-deoxyguanosine levels in the Fisher 344 rat. Nutr Cancer 40:125–133.

Hecht SS. 1999. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. J Nutr 129: 768–774.

Hecht SS, Kenney PM, Wang M, Trushin N, Upadhyaya P. 2000. Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/J mice by benzo[a]pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Lett 150:49–56.

Hecht SS, Huang C, Stoner GD, Li J, Kenney PM, Sturla SJ, Carmella SG. 2006. Identification of cyanidin glycosides as constituents of freeze-dried black raspberries which inhibit anti-benzo[a]pyrene-7,8-diol-9,10-epoxide-induced NFkappaB and AP-1 activity. Carcinogenesis 27: 1617–1626.

Herman-Antosiewicz A, Singh SV. 2005. Checkpoint kinase 1 regulates diallyl trisulfide-induced mitotic arrest in human prostate cancer cells. J Biol Chem 280:28519–28528.

Herman-Antosiewicz A, Johnson DE, Singh SV. 2006. Sulforaphane causes autophagy to inhibit release of cytochrome *c* and apoptosis in human prostate cancer cells. Cancer Res 66:5828–5835.

Herman-Antosiewicz A, Powolny AA, Singh SV. 2007a. Molecular targets of cancer chemoprevention by garlic-derived organosulfides. Acta Pharmacol Sin 28:1355–1364.

Herman-Antosiewicz A, Stan SD, Hahm ER, Xiao D, Singh SV. 2007b. Activation of a novel ataxia-telangiectasia mutated and Rad3 related/checkpoint kinase 1-dependent prometaphase checkpoint in cancer cell by diallyl trisulfide, a promising cancer chemopreventive constituent of processed garlic. Mol Cancer Ther 6:1249–1261.

Herman-Antosiewicz A, Xiao H, Lew KL, Singh SV. 2007c. Induction of p21 protein protects against sulforaphane-induced mitotic arrest in LNCaP human prostate cancer cell line. Mol Cancer Ther 6:1673–1681.

Higdon JV, Delage B, Williams DE, Dashwood RH. 2007. Cruciferous vegetables and human cancer risk: Epidemiological evidence and mechanistic basis. Pharmacol Res 55:224–236.

Hosono T, Fukao T, Ogihara J, Ito Y, Shiba H, Seki T, Ariga T. 2005. Diallyl trisulfide suppresses the proliferation and induces apoptosis of human colon cancer cells through oxidative modification of beta-tubulin. J Biol Chem 280:41487–41493.

Hsing AW, Chokkalingam AP, Gao YT, Madigan MP, Deng J, Gridley G, Fraumeni JF, Jr. 2002. *Allium* vegetables and risk of prostate cancer: A population-based study. J Natl Cancer Inst 94:1648–1651.

Hu X, Benson PJ, Srivastava SK, Mack LM, Xia H, Gupta V, Zaren HA, Singh SV. 1996. Glutathione S-transferases of female A/J mouse liver and forestomach and their differential induction by anti-carcinogenic organosulfides from garlic. Arch Biochem Biophys 336:199–214.

Hu R, Hebbar V, Kim BR, Chen C, Winnik B, Buckley B, Soteropoulos P, Tolias P, Hart RP, Kong AN. 2004. In vivo pharmacokinetics and regulation of gene expression profiles by isothiocyanate sulforaphane in the rat. J Pharmacol Exp Ther 310:263–271.

Hu R, Khor TO, Shen G, Jeong WS, Hebbar V, Chen C, Xu C, Reddy B, Chada K, Kong AN. 2006. Cancer

chemoprevention of intestinal polyposis in ApcMin/+ mice by sulforaphane, a natural product derived from cruciferous vegetable. Carcinogenesis 27:2038–2046.

Hu J, Straub J, Xiao D, Singh SV, Yang HS, Sonenberg N, Vatsyayan J. 2007. Phenethyl isothiocyanate, a cancer chemopreventive constituent of cruciferous vegetables, inhibits cap-dependent translation by regulating level and phosphorylation of 4E-BP1. Cancer Res 67:3569–3573.

Hussain SP, Jannu LN, Rao AR. 1990. Chemopreventive action of garlic on methylcholanthrene-induced carcinogenesis in the uterine cervix of mice. Cancer Lett 49:175–180.

Imada O. 1990. Toxicity aspects of garlic. First world congress on the health significance of garlic and garlic constituents. Washington, DC, August 28–30.

Jackson SJ, Singletary KW. 2004. Sulforaphane: A naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. Carcinogenesis 25:219–227.

Jackson SJ, Singletary KW, Venema RC. 2007. Sulforaphane suppresses angiogenesis and disrupts endothelial mitotic progression and microtubule polymerization. Vasc Pharmacol 46:77–84.

Ji Y, Morris ME. 2003. Determination of phenethyl isothiocyanate in human plasma and urine by ammonia derivatization and liquid chromatography-tandem mass spectrometry. Anal Biochem 323:39–47.

Ji Y, Kuo Y, Morris ME. 2005. Pharmacokinetics of dietary phenethyl isothiocyanate in rats. Pharmaceut Res 22:1658–1666.

Kelloff GJ. 2000. Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 78:199–334.

Kim H, Kim EH, Eom YW, Kim WH, Kwon TK, Lee SJ, Choi KS. 2006. Sulforaphane sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant hepatoma cells to TRAIL-induced apoptosis through reactive oxygen species-mediated up-regulation of DR5. Cancer Res 66:1740–1750.

Kim YA, Xiao D, Xiao H, Powolny AA, Lew KL, Reilly ML, Zeng Y, Wang Z, Singh SV. 2007. Mitochondria-mediated apoptosis by diallyl trisulfide in human prostate cancer cells is associated with generation of reactive oxygen species and regulated by Bax/Bak. Mol Cancer Ther 6:1599–1609.

Knowles LM, Milner JA. 2000. Diallyl disulfide inhibits p34(cdc2) kinase activity through changes in complex formation and phosphorylation. Carcinogenesis 21: 1129–1134.

Kresty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, Blackwood M, Stoner GD. 2001. Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. Cancer Res 61:6112–6119.

Kresty LA, Frankel W, Hammond C, Baird M, Mele JM, Stoner GD, Fromkes J. 2006. Transitioning from preclinical to clinical chemopreventive assessments of lyophilized black raspberries: Interim results show berries modulate markers of oxidative stress in Barrett's esophagus patients. Nutr Cancer 54:148–156.

Lampe JW, Peterson S. 2002. Brassica, biotransformation and cancer risk: Genetic polymorphisms alter the preventive effects of cruciferous vegetables. J Nutr 132: 2991–2994.

Li H, Li HQ, Wang Y, Xu HX, Fan WT, Wang ML, Sun PH, Xie XY. 2004. An intervention study to prevent gastric cancer by micro-selenium and large dose of allitridum. Chin Med J 117:1155–1160.

Li N, Guo R, Li W, Shao J, Li S, Zhao K, Chen X, Xu N, Liu S, Lu Y. 2006. A proteomic investigation into a human gastric cancer cell line BGC823 treated with diallyl trisulfide. Carcinogenesis 27:1222–1231.

Liebes L, Conaway CC, Hochster H, Mendoza S, Hecht SS, Crowell J, Chung FL. 2001. High performance liquid chromatography-based determination of total isothiocyanate levels in human plasma: Application to studies with 2-phenethyl isothiocyanate. Anal Biochem 291:279–289.

Lin JG, Chen GW, Su CC, Hung CF, Yang CC, Lee JH, Chung JG. 2002. Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine N-acetyltransferase activity and 2-aminofluorene-DNA adducts in human promyelocytic leukemia cells. Am J Chin Med 30:315–325.

Liu RH. 2003. Health benefits of fruit and vegetables and from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78:517s–520s.

London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK, Yu MC. 2000. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung cancer risk: A prospective study of men in Shanghai, China. Lancet 356:724–729.

Lu H, Li J, Zhang D, Stoner GD, Huang C. 2006. Molecular mechanisms involved in chemoprevention of black raspberry extracts: From transcription factors to their target genes. Nutr Cancer 54:69–78.

Lubet RA, Steele VE, Eto I, Juliana MM, Kelloff GJ, Grubbs CJ. 1997. Chemopreventive efficacy of anethole trithione, *N*-acetyl-L-cysteine, micanozole and phenethylisothiocyanate in the DMBA-induced rat mammary cancer model. Int J Cancer 72:95–101.

Mandal S, Stoner GD. 1990. Inhibition of *N*-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats by ellagic acid. Carcinogenesis 11:55–61.

Mandal S, Shivapurkar NM, Galati AJ, Stoner GD. 1988. Inhibition of *N*-nitrosomethylbenzylamine metabolism and DNA binding in cultured rat esophagus by ellagic acid. Carcinogenesis 9:1313–1316.

Michels KB, Giovannucci E, Joshipura KJ, Rosner BA, Stampfer MJ, Fuchs CS, Colditz GA, Speizer FE, Willett WC. 2000. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. J Natl Cancer Inst 92:1740–1752.

Miller AB, Altenburg HP, Bueno-de-Mesquita B, Boshuizen HC, Agudo A, Berrino F, Gram IT, Janson L, Linseisen J, Overvad K, Rasmuson T, Vineis P, Lukanova A, Allen N, Amiano P, Barricarte A, Berglund G, Boeing H, Clavel-Chapelon F, Day NE, Hallmans G, Lund E, Martinez C, Navarro C, Palli D, Panico S, Peeters PH, Quiros JR, Tjonneland A, Tumino R, Trichopoulou A, Trichopoulos D, Slimani N, Riboli E. 2004. Fruits and vegetables and lung cancer: Findings from the European prospective investigation into cancer and nutrition. Int J Cancer 108: 269–276.

Milner JA. 2004. Molecular targets for bioactive food components. J Nutr 134:2492s–2498s.

Miyoshi N, Uchida K, Osawa T, Nakamura Y. 2004. A link between benzyl isothiocyanate-induced cell cycle arrest and apoptosis: Involvement of mitogen-activated protein

kinases in the Bcl-2 phosphorylation. Cancer Res 64: 2134–2142.
Morse MA, Amin SG, Hecht SS, Chung FL. 1989. Effects of aromatic isothiocyanates on tumorigenicity, O^6-methylguanine formation, and metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. Cancer Res 49:2894–2897.
Morse MA, Eklind KI, Hecht SS, Jordan KG, Choi CI, Desai DH, Amin SG, Chung FL. 1991. Structure-activity relationships for inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone lung tumorigenesis by arylalkyl isothiocyanates in A/J mice. Cancer Res 51:1846–1850.
Mukhtar H, Das M, Del Tito BJ, Jr., Bickers DR. 1984. Protection against 3-methylcholanthrene-induced skin tumorigenesis in Balb/c mice by ellagic acid. Biochem Biophys Res Commun 119:751–757.
Myzak MC, Karplus PA, Chung FL, Dashwood RH. 2004. A novel mechanism of chemoprotection by sulforaphane: Inhibition of histone deacetylase. Cancer Res 64:5767–5774.
Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E. 2006. Sulforaphane inhibits histone deacetylase activity in BPH-1, LNCaP and PC-3 prostate epithelial cells. Carcinogenesis 27:811–819.
Nagae S, Ushijima M, Hatono S, Imai J, Kasuga S, Matsuura H, Itakura Y, Higashi Y. 1994. Pharmacokinetics of the garlic compound S-allyl cysteine. Planta Med 60:214–217.
Nakamura Y, Kawakami M, Yoshihiro A, Miyoshi N, Ohigashi H, Kawai K, Osawa T, Uchida K. 2002. Involvement of the mitochondrial death pathway in chemopreventive benzyl isothiocyanate-induced apoptosis. J Biol Chem 277:8492–8499.
Narayanan BA, Geoffroy O, Willingham MC, Re GG, Nixon DW. 1999. p53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett 136:215–221.
Nishikawa A, Furukawa F, Uneyama C, Ikezaki S, Tanakamaru Z, Chung FL, Takahashi M, Hayashi Y. 1996. Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in N-nitrosobis(2-oxopropyl)amine-treated hamsters. Carcinogenesis 17:1381–1384.
Nishikawa T, Yamada N, Hattori A, Fukuda H, Fujino T. 2002. Inhibition by ajoene of skin-tumor promotion in mice. Biosci Biotechnol Biochem 66:2221–2223.
Rao CV, Tokumo K, Rigotty J, Zang E, Kelloff G, Reddy BS. 1991. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, alpha-difluoromethylornithine, 16 alpha-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res 51: 4528–4534.
Reddy BS, Rao CV, Rivenson A, Kelloff G. 1993. Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res 53:3493–3496.
Reen RK, Nines R, Stoner GD. 2006. Modulation of *N*-nitrosomethylbenzylamine metabolism by black raspberries in the esophagus and liver of Fisher 344 rats. Nutr Cancer 54:47–54.
Robert V, Mouille B, Mayeur C, Michaud M, Blachier F. 2001. Effects of the garlic compound diallyl disulfide on the metabolism, adherence and cell cycle of HT-29 colon carcinoma cells: Evidence of sensitive and resistant subpopulations. Carcinogenesis 22:1155–1161.
Ross HA, McDougall GJ, Stewart D. 2007. Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. Phytochem 68:218–228.
Schaffer EM, Liu JZ, Green J, Dangler CA, Milner JA. 1996. Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea-induced rat mammary carcinogenesis. Cancer Lett 102:199–204.
Seki T, Tsuji K, Hayato Y, Moritomo T, Ariga T. 2000. Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. Cancer Lett 160:29–35.
Seow A, Yuan JM, Sun CL, Van Den Berg D, Lee HP, Yu MC. 2002. Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. Carcinogenesis 23: 2055–2061.
Shen G, Xu C, Chen C, Hebbar V, Kong AN. 2006. p53-independent G_1 cell cycle arrest of human colon carcinoma cells HT-29 by sulforaphane is associated with induction of $p21^{CIP1}$ and inhibition of expression of cyclin D1. Cancer Chemother Pharmacol 57:317–327.
Shukla Y, Kalra N. 2007. Cancer chemoprevention with garlic and its constituents. Cancer Lett 247:167–181.
Shumway BS, Kresty LA, Larsen PE, Zwick JC, Lu B, Fields HW, Mumper RJ, Stoner GD, Mallery SR. 2007. Effects of a topically applied bioadhesive berry gel on loss of heterozygosity indices in premalignant oral lesions. (submitted for publication).
Siegers CP. 1992. Allium sativum. In: De Smet PAGM, Keller K, Hansel R, Chandler RF, editors. Adverse effects of herbal drugs. Berlin: Springer-Verlag. pp 73–77.
Singh A, Shukla Y. 1998. Antitumor activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. Cancer Lett 131:209–214.
Singh SV, Mohan RR, Agarwal R, Benson PJ, Hu X, Rudy MA, Xia H, Katoh A, Srivastava SK, Mukhtar H, Gupta V, Zaren HA. 1996. Novel anti-carcinogenic activity of an organosulfide from garlic: Inhibition of H-RAS oncogene transformed tumor growth in vivo by diallyl disulfide is associated with inhibition of $p21^{H\text{-}ras}$ processing. Biochem Biophys Res Commun 225:660–665.
Singh SV, Mack LM, Xia H, Srivastava SK, Hu X, Benson PJ, Murthy M, McFadden E, Gupta V, Zaren HA. 1997. Differential induction of glutathione redox-cycle enzymes by anti-carcinogenic organosulfides from garlic. Clin Chem Enzymol Commun 7:287–297.
Singh SV, Pan SS, Srivastava SK, Xia H, Hu X, Zaren HA, Orchard JL. 1998. Differential induction of NAD(P)H:-quinone oxidoreductase by anti-carcinogenic organosulfides from garlic. Biochem Biophys Res Commun 244: 917–920.
Singh A, Arora A, Shukla Y. 2004a. Modulation of altered hepatic foci induction by diallyl sulphide in Wistar rats. Eur J Cancer Prev 13:263–269.
Singh AV, Xiao D, Lew KL, Dhir R, Singh SV. 2004b. Sulforaphane induces caspase-mediated apoptosis in cultured PC-3 human prostate cancer cells and retards growth of PC-3 xenografts in vivo. Carcinogenesis 25:83–90.
Singh SV, Herman-Antosiewicz A, Singh AV, Lew KL, Srivastava SK, Kamath R, Brown KD, Zhang L, Baskaran R. 2004c. Sulforaphane-induced G2/M phase cell cycle arrest involves checkpoint kinase 2-mediated

phosphorylation of Cdc25C. J Biol Chem 279:25813–25822.
Singh SV, Srivastava SK, Choi S, Lew KL, Antosiewicz J, Xiao D, Zeng Y, Watkins SC, Johnson CS, Trump DL, Lee YJ, Xiao H, Herman-Antosiewicz A. 2005. Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. J Biol Chem 280: 19911–19924.
Sparnins VL, Barany G, Wattenberg LW. 1988. Effects of organosulfur compounds from garlic and onions on benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. Carcinogenesis 9: 131–134.
Srivastava SK, Singh SV. 2004. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. Carcinogenesis 25:1701–1709.
Stoner GD, Morrissey DT, Heur YH, Daniel EM, Galati AJ, Wagner SA. 1991. Inhibitory effects of phenethyl isothiocyanate on *N*-nitrosobenzylmethylamine carcinogenesis in the rat esophagus. Cancer Res 51:2063–2068.
Stoner GD, Sardo C, Apseloff G, Mullet D, Wargo W, Pound V, Singh A, Sanders J, Aziz R, Casto B, Sun X. 2005. Pharmacokinetics of anthocyanins and ellagic acid in healthy volunteers fed freeze-dried black raspberries daily for 7 days. J Clin Pharmacol 45:1153–1164.
Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. 2006. Protection against esophageal cancer in rodents with lyophilized berries: Potential mechanism. Nutr Cancer 54:33–46.
Stoner GD, Wang L-S, Zikri N, Chen T, Hecht SS, Huang C, Sardo C, Lechner JF. 2007. Cancer prevention with freeze-dried berries and berry components. Semin Cancer Biol 17:403–410.
Sun X, Guo T, He J, Zhao M, Yan M, Cui F, Deng Y. 2006. Determination of the concentration of diallyl trisulfide in rat whole blood using gas chromatography with electron-capture detection and identification of its major metabolite with gas chromatography mass spectrometry. Yakugaku Zasshi 126:521–527.
Sundaram SG, Milner JA. 1996. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 17:669–673.
Surh YJ. 2003. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780.
Suzui N, Sugie S, Rahman KM, Ohnishi M, Yoshimi N, Wakabayashi K, Mori H. 1997. Inhibitory effects of diallyl disulfide or aspirin on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats. Jpn J Cancer Res 88:705–711.
Takahashi S, Hakoi K, Yada H, Hirose M, Ito N, Fukushima S. 1992. Enhancing effects of diallyl sulfide on hepatocarcinogenesis and inhibitory actions of the related diallyl disulfide on colon and renal carcinogenesis in rats. Carcinogenesis 13:1513–1518.
Tanaka S, Haruma K, Yoshihara M, Kajiyama G, Kira K, Amagase H, Chayama K. 2006. Aged garlic extract has potential suppressive effect on colorectal adenomas in humans. J Nutr 136:821s–826s.
Tang L, Zhang Y. 2005. Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol Cancer Ther 4:1250–1259.
Thejass P, Kuttan G. 2006. Immunomodulatory activity of Sulforaphane, a naturally occurring isothiocyanate from broccoli (Brassica oleracea). Phytomedicine 14:538–545.
Thejass P, Kuttan G. 2007. Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) inhibit tumour-specific angiogenesis by downregulating nitric oxide (NO) and tumour necrosis factor-alpha (TNF-alpha) production. Nitric Oxide 16:247–257.
Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P. 2006. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell 10:241–252.
Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, van den Brandt PA. 1996. Epidemiological studies on Brassica vegetables and cancer risk. Cancer Epidemiol Biomark Prev 5:733–748.
Visanji JM, Duthie SJ, Pirie L, Thompson DG, Padfield PJ. 2004. Dietary isothiocyanates inhibit Caco-2 cell proliferation and induce G2/M phase cell cycle arrest, DNA damage, and G2/M checkpoint activation. J Nutr 134: 3121–3126.
Voorrips LE, Goldbohm RA, van Poppel G, Sturmans F, Hermus RJ, van den Brandt PA. 2000. Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study: The Netherlands Cohort Study on Diet and Cancer. Am J Epidemiol 152:1081–1092.
Wang L, Liu D, Ahmed T, Chung FL, Conaway C, Chiao JW. 2004. Targeting cell cycle machinery as a molecular mechanism of sulforaphane in prostate cancer prevention. Int J Oncol 24:187–192.
Wargovich MJ, Woods C, Eng VW, Stephens LC, Gray K. 1988. Chemoprevention of *N*-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. Cancer Res 48: 6872–6875.
Wargovich MJ, Chen CD, Jimenez A, Steele VE, Velasco M, Stephens LC, Price R, Gray K, Kelloff GJ. 1996. Aberrant crypts as a biomarker for colon cancer: Evaluation of potential chemopreventive agents in the rat. Cancer Epidemiol Biomarkers Prev 5:355–360.
Wattenberg LW. 1987. Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo[a]pyrene on pulmonary and forestomach neoplasia in A/J mice. Carcinogenesis 8:1971–1973.
Wilkinson JT, Morse MA, Kresty LA, Stoner GD. 1995. Effect of alkyl chain length on inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis and DNA methylation by isothiocyanates. Carcinogenesis 16:1011–1015.
Xiao D, Singh SV. 2006. Diallyl trisulfide, a constituent of processed garlic, inactivates Akt to trigger mitochondrial translocation of BAD and caspase-mediated apoptosis in human prostate cancer cells. Carcinogenesis 27:533–540.
Xiao D, Singh SV. 2007. Phenethyl isothiocyanate inhibits angiogenesis *in vitro* and *ex vivo*. Cancer Res 67:2239–2246.
Xiao D, Srivastava SK, Lew KL, Zeng Y, Hershberger P, Johnson CS, Trump DL, Singh SV. 2003. Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing

G2/M arrest and inducing apoptosis. Carcinogenesis 24:891–897.

Xiao D, Choi S, Johnson DE, Vogel VG, Johnson CS, Trump DL, Lee YJ, Singh SV. 2004a. Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. Oncogene 23: 5594–5606.

Xiao D, Johnson CS, Trump DL, Singh SV. 2004b. Proteasome-mediated degradation of cell division cycle 25C and cyclin-dependent kinase 1 in phenethyl isothiocyanate-induced G2/M phase cell cycle arrest in PC-3 human prostate cancer cells. Mol Cancer Ther 3:567–575.

Xiao D, Herman-Antosiewicz A, Antosiewicz J, Xiao H, Brisson M, Lazo JS, Singh SV. 2005a. Diallyl trisulfide-induced G2-M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species-dependent destruction and hyperphosphorylation of Cdc25C. Oncogene 24:6256–6268.

Xiao D, Zeng Y, Choi S, Lew KL, Nelson JB, Singh SV. 2005b. Caspase-dependent apoptosis induction by phenethyl isothiocyanate, a cruciferous vegetable-derived cancer chemopreventive agent, is mediated by Bak and Bax. Clin Cancer Res 11:2670–2679.

Xiao D, Lew KL, Kim YA, Zeng Y, Hahm ER, Dhir R, Singh SV. 2006a. Diallyl trisulfide suppresses growth of PC-3 human prostate cancer xenograft *in vivo* in association with Bax and Bak induction. Clin Cancer Res 12:6836–6843.

Xiao D, Li M, Herman-Antosiewicz A, Antosiewicz J, Xiao H, Lew KL, Zeng Y, Marynowski SW, Singh SV. 2006b. Diallyl trisulfide inhibits angiogenic features of human umbilical vein endothelial cells by causing Akt inactivation and down-regulation of VEGF and VEGF-R2. Nut Cancer 55:94–107.

Xiao D, Lew KL, Zeng Y, Xiao H, Marynowski SW, Dhir R, Singh SV. 2006c. Phenethyl isothiocyanate-induced apoptosis in PC3 human prostate cancer cells is mediated by reactive oxygen species-dependent disruption of the mitochondrial membrane potential. Carcinogenesis 27: 2223–2234.

Xiao D, Vogel V, Singh SV. 2006d. Benzyl isothiocyanate-induced apoptosis in human breast cancer cells is initiated by reactive oxygen species and regulated by Bax and Bak. Mol Cancer Ther 5:2931–2945.

Xu B, Monsarrat B, Gairin JE, Girbal-Neuhauser E. 2004. Effect of ajoene, a natural antitumor small molecule, on human 20S proteasome activity in vitro and in human leukemic HL60 cells. Fundam Clin Pharmacol 18:171–180.

Xu C, Shen G, Chen C, Gelinas C, Kong AN. 2005. Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. Oncogene 24:4486–4495.

Ye L, Zhang Y. 2001. Total intracellular accumulation levels of dietary isothiocyanates determine their activity in elevation of cellular glutathione and induction of Phase 2 detoxification enzymes. Carcinogenesis 22: 1987–1992.

You WC, Blot WJ, Chang YS, Ershow A, Yang ZT, An Q, Henderson BE, Fraumeni JF, Jr., Wang TG. 1989. *Allium* vegetables and reduced risk of stomach cancer. J Natl Cancer Inst 81:162–164.

Zhang K, Zuo Y. 2004. GC-MS determination of flavonoids and phenolic and benzoic acids in human plasma after consumption of cranberry juice. J Agric Food Chem 52: 222–227.

Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P. 1994. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci USA 91:3147–3150.

Zhang Y, Tang L, Gonzalez V. 2003. Selected isothiocyanates rapidly induce growth inhibition of cancer cells. Mol Cancer Ther 2:1045–1052.