Sulforaphane Glucosinolate Monograph

Introduction

Intake of broccoli sprouts, a rich source of the glucosinolate glucoraphanin, has been associated with decreased incidence, multiplicity, and tumor growth in animal cancer models.[1-3] In 1992, Paul Talalay, MD, and colleagues at Johns Hopkins University identified the isothiocyanate, sulforaphane, a biologically active metabolite of glucoraphanin, as the compound in broccoli responsible for many of its health benefits.[4] Since that time, more than 500 studies have been conducted on the mechanisms and biological activity of sulforaphane and its precursor, glucoraphanin.[5] Glucoraphanin, also referred to as sulforaphane glucosinolate (SGS), is the most potent naturally-occurring inducer of phase 2 detoxification enzymes[4,6] and is an indirect, long-acting antioxidant.[7-9] Sulforaphane also exhibits broad-spectrum antimicrobial activity against numerous gram-positive and -negative bacteria,[10] most notably *Helicobacter pylori*.[11] In addition, sulforaphane possesses anti-inflammatory activity; it inhibits cytokine production in preclinical and clinical studies.[12-14] Sulforaphane's multiple molecular targets and promising early research have led to 15 clinical trials currently underway to assess its effects on various cancers, cardiovascular disease, upper airway inflammation, radiation dermatitis, and vascular health.[15]

Biochemistry

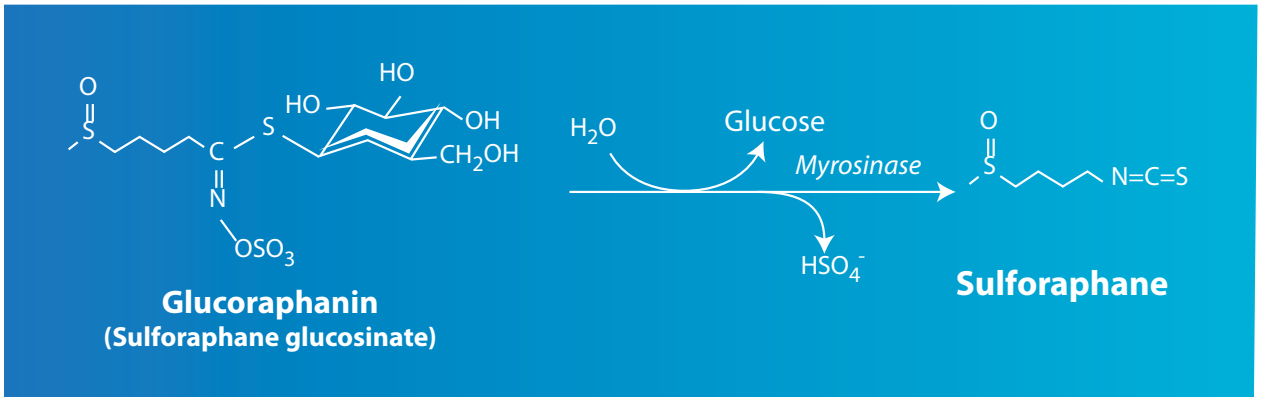
Glucoraphanin is a glucosinolate found in high concentrations in the Mariner variety of broccoli (*Brassica oleracea italica*) and other members of the Brassica family.[16] All glucosinolates are comprised of a basic structure consisting of a β-D-thioglucose group, a sulphonated oxime group, and an amino acid-derived side chain.[17] Glucosinolates must be enzymatically hydrolyzed to their associated isothiocyanate to become active.[18] Sulforaphane (molecular formula $C_6H_{11}NOS_2$) is the biologically active isothiocyanate produced when glucoraphanin is metabolized by the enzyme myrosinase (Figure 1).[19]

Pharmacokinetics

Glucoraphanin in broccoli is enzymatically hydrolyzed by myrosinase, an enzyme compartmentally separated from glucoraphanin in plant cells. Myrosinase is released when the plant is chewed or processed.[20] Heating broccoli partially denatures and inactivates myrosinase, leaving the glucoraphanin at least partially intact. In the gut of healthy individuals any intact glucoraphanin is then metabolized by myrosinase-producing bacteria.[21] Because broccoli sprout or seed extracts taken orally contain no myrosinase to hydrolyze the glucoraphanin, transformation to sulforaphane must be carried out by the gut microflora.[22] In individuals with compromised intestinal flora and low myrosinase activity, it is unclear if glucoraphanin exerts the same systemic effects as observed in individuals with normal intestinal flora.[23]

Research in humans indicates approximately 74 percent of sulforaphane from broccoli extract is absorbed in the jejunum.[24] After absorption, sulforaphane is metabolized via the mercapturic acid pathway.[25,26] Although this pathway involves a complex interplay between the liver, small

Figure 1. Glucoraphanin Metabolism



intestine, and kidneys, the liver is thought to be the primary site of activity and is the site of sulforaphane conjugation to glutathione. Sulforaphane-glutathione conjugates are subsequently converted to cysteinyl-glycine, cysteine, and N-acetylcysteine conjugates in the kidneys or gut and then cycled back to the liver for acetylation. Of these conjugates, sulforaphane-N-acetylcysteine is the most prevalent.[21]

Upon absorption into the bloodstream, sulforaphane readily accumulates in tissue and exerts anticarcinogenic effects. In one human study, a single 200 µM dose of sulforaphane from broccoli sprouts yielded peak plasma concentrations between 0.943 and 2.27 µmol/L at one hour post feeding; the half life of sulforaphane was 1.77 ± 0.13 hours.[27] A pilot study in eight healthy women undergoing reduction mammoplasty demonstrated a broccoli sprout extract containing 200 µM sulforaphane given orally one hour prior to tissue removal resulted in average tissue uptake of 1.45 ± 1.12 pmol/mg in the left breast and 2.00 ± 1.95 pmol/mg in the right breast. Both detoxification enzyme genes for NADH quinone reductase (NQO1) and heme oxygenase-1 (HO-1) were measureable in the excised breast tissue, indicating cancer blocking activity after sulforaphane consumption.[28] Research in mice has also demonstrated colonic tissue uptake of sulforaphane after oral dosing, accompanied by a reduction in adenoma formation.[29] Excretion of sulforaphane conjugates in the urine is via first-order kinetics with metabolites being cleared from the body within 72 hours of dosing.[27,30]

Key words: sulforaphane, broccoli, glucoraphanin, SGS, isothiocyanate, Crucera, detoxification, detox, phase 2, phase II, H. pylori, Helicobacter, Brassica, cruciferous, chemoprotection, chemoprotectant, anticarcinogen, carcinogen, cancer, inflammation, Gilbert's syndrome, anti-inflammatory, antioxidant, Nrf2

Mechanisms of Action

Indirect Antioxidant and Carcinogen Detoxification

Sulforaphane is a pleotropic molecule and a potent inducer of numerous nuclear factor erythroid-derived 2 (Nrf2)-dependent phase 2 enzymes involved in xenobiotic detoxification. Enzymes induced by sulforaphane include the antioxidant response element (ARE) targets: NQO1,[4] γ-glutamylcysteine synthetase (GGCS),[31] HO-1,[32] glutathione transferases (GST),[33] glucuronosyl transferases,[34] and epoxide hydrolases.[35] These enzymes are regulated by the Nrf2 transcription factor, which upon release from the Kelch-like ECH-associated protein 1 (KEAP1), binds to ARE sites in the enzymes' genes and upregulates carcinogen detoxification.[36,37] Other Nrf2-mediated effects of sulforaphane include inhibition of LDL oxidation,[38] inhibition of dopamine oxidation,[39] improvement of age-related TH1 immunity via restoration of redox equilibrium,[40] and reduction of oxidative stress caused by electrophilic carcinogens.[41] Sulforaphane also modulates phase 1 cytochrome p450 (CYP) enzymes by decreasing CYP1A1, CYP2B1/2, and CYP3A4 activity, thereby inhibiting the activation of procarcinogens and preventing the generation of DNA adducts during the initiation stage of cancer.[42] The overall net effect on phase 1 and 2 enzymes is an increase in metabolism and detoxification of chemical carcinogens.[43]

Other Chemopreventive Mechanisms

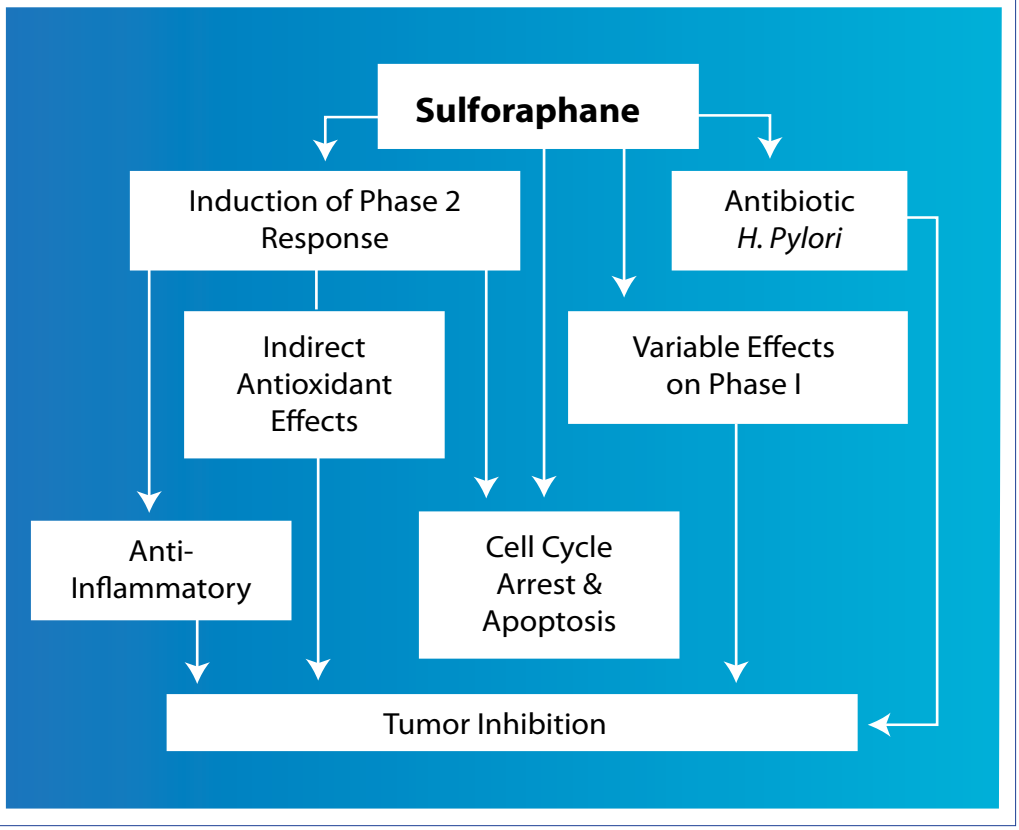
Sulforaphane exerts a direct effect on human cancer cells post-initiation. Research has demonstrated sulforaphane directly inhibits cell cycle progression, primarily via G_2M arrest,[44,45] and induces apoptosis of cancer cells via caspase

activation, resulting in reduced tumor weight and volume both *in vitro* and in animal cancer models.[45,46] In human tissue samples, reductions in histone acetylation correlate with increased cancer grade and risk of cancer recurrence.[47] Studies show sulforaphane directly inhibits histone deacetylase (HDAC), which correlates with induction of G_2M cell cycles arrest and apoptosis.[48] Sulforaphane also appears to upregulate apoptosis in cancer cells by modulating nuclear factor kappaB (NFκB) activity[49] and increasing mitochondrial reactive oxygen species, causing disruption of mitochondrial membrane potential and release of cytochrome C.[50] And finally, sulforaphane potently inhibits angiogenesis and metastasis of tumors by reducing microcapillary formation and inhibiting cell migration.[51] These effects were associated with down regulation of angiogenesis factors, including vascular endothelial growth factor (VEGF).[52] Figure 2 summarizes the tumor inhibition effects of sulforaphane.

Miscellaneous Mechanisms

Sulforaphane's anti-inflammatory effects have been attributed to inhibition of pro-inflammatory signaling molecules and cytokines[13] such as NFκB, prostaglandin E2, and nitric oxide.[12] Sulforaphane also appears to reduce upper airway inflammation via increased phase 2 enzyme detoxification of air pollutants and pollen, apparently via decreased cellular oxidative stress, inhibition of inflammatory cytokine production, and decreased tissue inflammation.[14] *In vitro* research has also shown sulforaphane inhibits the production of interleukin and tumor necrosis factor-alpha (TNF-α) in rheumatoid T cells.[53] Sulforaphane exhibits broad-spectrum antimicrobial activity, inhibiting the growth of several gram-positive and -negative bacteria, including *E. coli* 0157:H7, *Helicobacter pylori*, Salmonella, Shigella, *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa,* and *Cryptococcus neoformans*.[10,11]

Figure 2. Sulforaphane Mechanisms of Tumor Inhibition



Clinical Indications

Cancer

Preclinical and Animal Research

Numerous *in vitro* studies in human colon, leukemia, pancreatic, lung, and skin cancer cell lines have demonstrated sulforaphane's inhibitory effects on cell cycle arrest,[45,54-56] and research in human bladder[57] and prostate[46] cell lines has shown it increases apoptosis. Sulforaphane's ability to disrupt tubulin polymerization and inhibit mitosis has also been demonstrated in animal models of breast cancer.[58,59] Inhibition of histone deacetylase and increased apoptosis in human colon, prostate, and kidney cell lines has also been reported.[48,60,61]

In a pilot study involving three healthy volunteers (ages 18-55), a single daily dose of 68 g BroccoSprouts® (approximately 105 mg sulforaphane) significantly inhibited HDAC activity in peripheral blood mononuclear cell cultures three and six hours following consumption, suggesting sulforaphane may induce cell cycle arrest and apoptosis in humans.[62]

In mice with experimentally induced prostate cancer, 6 µmol sulforaphane by oral gavage three times weekly from age six weeks onward decreased pulmonary metastasis incidence by 50 percent and multiplicity by 63 percent. Prostate tissue samples revealed decreased cellular proliferation and increased apoptosis when compared to control mice.[63] Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in a wide variety of cancer cells. In a mouse model of prostate cancer, tumor-bearing male mice were given sulforaphane (40 mg/kg), TRAIL (15 mg/kg) + sulforaphane (40 mg/kg), TRAIL alone (15 mg/kg), or vehicle at varying intervals for four weeks. Although either sulforaphane or TRAIL alone decreased tumor growth, the combination of sulforaphane and TRAIL was more effective, suggesting sulforaphane may have a potentiating effect on TRAIL. The sulforaphane-TRAIL combination also activated several caspases and was more effective at inhibiting markers of angiogenesis and metastasis than either agent alone.[64] Sulforaphane given to female breast cancer-bearing, non-obese, diabetic/severe combined immunodeficient (NOD/SCID) mice at a daily dose of 50 mg/kg for two weeks eliminated breast cancer stem cells *in vivo* and halted tumor growth.[65]

Clinical Studies

The first direct observation of sulforaphane's inhibitory effect on cancer in humans was observed in 200 healthy adults (ages 25-65) from the Jiangsu Province of China, a region with a high rate of hepatocellular carcinoma due to excessive dietary aflatoxin exposure and chronic hepatitis B infection. The primary end-point of this blinded, placebo-controlled trial was to determine if drinking daily broccoli sprout infusions (containing 400 µmol glucoraphanin) for two weeks could reduce urinary excretion of aflatoxin DNA adducts – indicators of DNA damage. A highly significant inverse association was observed for excretion of dithiocarbamates (isothiocyanate metabolites of glucoraphanin) and aflatoxin-DNA adducts in individuals treated with broccoli sprout infusions. An average of approximately 12 percent (range 1-45 percent) of the administered dose of broccoli sprout glucoraphanin was excreted as dithiocarbamates, with significant variability in excretion rates. The reason for this variation may be due to differences in enteric microflora composition, some individuals possibly having less myrosinase. Genetic polymorphisms of the glutathione S-transferase enzyme involved in glucoraphanin metabolism may also be partially responsible.[66]

Cardiovascular Disease and Hypertension

Glucoraphanin and sulforaphane afford cardiovascular protection via their antioxidant and anti-inflammatory properties, resulting in reduced oxidative stress, improvement in lipid profiles, and decreased blood pressure. A phase 1 trial involving 12 cigarette smokers (six men and six women) investigated whether consuming 100 g fresh broccoli sprouts daily (glucoraphanin/sulforaphane content not specified) for one week impacted oxidative stress markers and cholesterol values. Cholesterol levels, plasma amino acids, natural killer cell activity, serum coenzyme Q10, and markers of oxidative stress – plasma phosphatidylcholine hydroperoxide (PCOOH), urinary 8-iso-prostane, and urinary 8-hydroxydeoxyguanosine – were measured pre- and post-treatment. After only one week of broccoli sprout intake, all subjects demonstrated decreased serum total- and LDL-cholesterol levels and reductions in all oxidative stress markers; females also had significantly increased HDL-cholesterol levels.[38]

Animal research supports these findings. Studies on male and female spontaneously hypertensive rats on a glucoraphanin-enriched diet (equivalent to 27.3 µmol sulforaphane per g dried sprouts)

showed decreased oxidative stress, lower blood pressure, and less renal and central nervous system inflammation in kidney and spinal cord tissue when compared to animals on glucoraphanin-free diets.[67,68]

Upper Airway Inflammation

Airborne diesel exhaust particles appear to exacerbate lung and cardiovascular diseases by inducing oxidative stress.[69] Sulforaphane inhibits cytokine production in human airway epithelial cells exposed to diesel extract via induction of phase 2 enzyme genes NQO1 and glutathione-S-transferase M1.[13] In the first study to demonstrate oral sulforaphane upregulation of phase 2 antioxidant enzyme expression in the human airway, Reidl et al administered BroccoSprouts® homogenates (BSH) to 57 healthy adult volunteers (average age 34) in a single-blind, dose-escalation (25, 50, 75, 100, 125, 150, 175, and 200 g), three-day trial. Analysis demonstrated a sulforaphane content of 0.283 µmol/mL BSH – the 175- and 200-mg doses delivering 89 and 102 µmol sulforaphane, respectively. Control subjects received a 200 g dose of alfalfa sprouts, containing negligible amounts of sulforaphane. Baseline nasal lavage and blood samples were collected from all participants and assessed for phase 2 enzyme expression. Subjects were assessed again one day after final dosing. Significant increases in glutathione-S-transferases, HO-1, and NQO1 were observed with the 200-g BSH dose compared to placebo. All doses were well tolerated and without serious side effects, although four subjects reported mild gastrointestinal events that did not require treatment.[14]

Helicobacter pylori Infection

The role of *Helicobacter pylori* in development of stomach cancer is well established.[70,71] Animal research shows sulforaphane given to human gastric xenograft-bearing mice at a daily dose of 1.33 mg (equivalent to a 100-mg daily dose in humans) is strongly bacteriocidal and eradicates *H. pylori*.[72] Yanaka et al subsequently demonstrated glucoraphanin-rich three-day old broccoli sprouts (6 µmol glucoraphanin/g) given to *H. pylori*-infected female mice reduced gastric bacterial colonization, decreased mucosal TNF-α and interleukin-1β expression, decreased gastric inflammation, and prevented gastric atrophy. These effects were not observed in Nrf2-depleted mice, indicating the important role of Nrf2-dependent phase 2 enzyme induction by sulforaphane.[73]

In a human arm of the Yanaka study, 48 *H. pylori*-infected patients were divided into a broccoli sprout treatment group (n=25) or an alfalfa sprout placebo group (n=23). Those in the broccoli sprout group received 70 g sprouts daily, containing 6 µmol glucoraphanin/g, for eight weeks. Glucoraphanin feeding decreased breath test urease levels, *H. pylori* antigen, and pepsinogens I and II – markers of gastric colonization and inflammation. These results indicate broccoli sprouts as a source of glucoraphanin improve *H. pylori* infection sequelae and enhance chemoprotection from *H. pylori*-induced stomach tumors.[73] Two other clinical trials demonstrated the bacteriocidal[74] and chemoprotective properties of sulforaphane in individuals with *H. pylori* infection.[75]

Gilbert's Syndrome

Gilbert's syndrome is characterized by genetic polymorphisms in the UDP-glucuronosyltransferase (UGT) enzymes, the primary one being UGT1A1*28, which is involved in bilirubin glucuronidation. UGT polymorphisms can manifest as benign unconjugated hyperbilirubinemia, associated with reduced hepatic conjugation, and may increase cancer risk in this population.[76] In an observational study of 191 nonsmoking volunteers (ages 19-40) consuming 0-4 servings of cruciferous vegetables daily, there was a statistically significant inverse association between the UGT1A1 gene/Cruciferae interaction and total, direct, and indirect bilirubin measurements. Sulforaphane from cruciferous vegetables has been shown to induce UGT1A1 activity, resulting in greater bilirubin conjugation and clearance and possibly mitigating the increased cancer risk.[77]

Rheumatoid Arthritis

Rheumatoid arthritis (RA) involves a tumor-like expansion of the synovium characterized by hyperproliferation of synoviocytes, infiltration of T and B cells, and increases in interleukin (IL) -6, -8, and -17. RA treatment involves suppression of synoviocyte proliferation and cytokine production.[78] Due to the "tumor-like" attributes of synoviocytes and their role in RA progression, Kong et al investigated the effect of sulforaphane on synoviocyte apoptosis in a mouse model of RA. Sulforaphane was administered peritoneally to male mice at concentrations of 12.8, 63.8, and 318.8 mg/mL/kg every other day for five weeks. Sulforaphane decreased synoviocyte survival up to 51 percent compared to baseline, significantly decreased IL-17 and TNF-α, and repressed the

proliferative response in polymorphonuclear cells to baseline levels. Histological examination revealed less inflammation, synovial hyperplasia, and bone destruction in mice treated with sulforaphane compared to the control group.[53]

Macular Degeneration

Oxidative stress in the retinal pigmented epithelial (RPE) cell layer is associated with age-related macular degeneration, the leading cause of blindness in the elderly.[79] *In vitro* and animal research demonstrates that sulforaphane protects RPE cells from photo-oxidative damage; the degree of protection correlated with basal levels of glutathione and NADH quinone reductase.[9,80]

Neurological Conditions

In vitro and animal research indicates sulforaphane treatment of various neuronal cell lines (neuroblastoma, astrocyte, and primary cortical neurons) protects against neuronal injury caused by oxidative stress and inflammation. This is accomplished via activation of Nrf2/ARE-mediated detoxification enzymes and results in increased intracellular glutathione levels and reduced rates of apoptosis.[81-84] These studies indicate sulforaphane may protect against the types of neuronal injury found in Parkinson's and Alzheimer's diseases.

Side Effects and Toxicity

Several studies have been conducted to assess the safety of sulforaphane in humans. A randomized, placebo-controlled, double-blind study showed broccoli sprout extracts were without significant side effects at doses of 25 and 100 µmol glucoraphanin for seven days.[85] Another randomized, placebo-controlled study involving 200 healthy adults consuming broccoli sprout infusions daily for two weeks (400 µmol or approximately 175 mg glucoraphanin) showed no adverse effects.[66] In a dose escalation safety study, broccoli sprout extracts containing sulforaphane doses as high as 340 nmol were topically applied three consecutive times to forearm skin. Researchers reported significant induction of phase II enzyme activity in biopsied tissue without any adverse reactions.[86]

Dosage

Based on available research, typical dosage for broccoli sprout and seed extracts is 50-100 mg sulforaphane glucosinolate daily in divided doses.

Warnings and Contraindications

Sulforaphane and glucoraphanin from broccoli, broccoli sprouts, and broccoli seeds has a good safety profile with no known contraindications or drug interactions.

References

1. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci* 1997;94:10367-10372.
2. Zhang Y, Kensler TW, Cho CG, et al. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc Natl Acad Sci* 1994;91:3147-3150.
3. Gerhauser C, You M, Liu J, et al. Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. *Cancer Res* 1997;57:272-278.
4. Zhang Y, Talayay P, Cho CG, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci* 1992;89:2399-2403.
5. http://www.brassica.com [Accessed October 2, 2010]
6. Prochaska HJ, Santamaria AB, Talalay P. Rapid detection of inducers of enzymes that protect against carcinogens. *Proc Natl Acad Sci* 1992;89:2394-2398.
7. Fahey JW, Talalay P. Antioxidant functions of sulforaphane: a potent inducer of phase II detoxification enzymes. *Food Chem Toxicol* 1999;37:973-979.
8. Zhang Y, Talalay P. Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res* 1994;54:1967s-1981s.
9. Tanito M, Masutani H, Kim YC, et al. Sulforaphane induces thioredoxin through the antioxidant-responsive element and attenuates retinal light damage in mice. *Invest Ophthalmol Vis Sci* 2005;46:979-987.
10. Johansson NL, Pavia CS, Chiao JW. Growth inhibition of a spectrum of bacterial and fungal pathogens by sulforaphane, an isothiocyanate product found in broccoli and other cruciferous vegetables. *Planta Med* 2008;74:747-750.
11. Fahey JW, Haristoy X, Dolan PM, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc Natl Acad Sci* 2002;99:7610-7615.
12. Heiss E, Herhaus C, Klimo K, et al. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem* 2001;276:32008-32015.

13. Ritz SA, Wan J, Diaz-Sanchez D. Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L33-L39.
14. Reidl MA, Saxon A, Diaz-Sanchez D. Oral sulforaphane increases phase II antioxidant enzymes in the human upper airway. *Clin Immunol* 2009;130:244-251.
15. www.clinicaltrials.gov. [Accessed: October 5, 2010.]
16. Vermeulen M, van der Berg R, Freidig AP, et al. Association between consumption of cruciferous vegetables and condiments and excretions in urine of isothiocyanate mercaptic acids. *J Agric Food Chem* 2006;54:5350-5358.
17. Keck AS, Finley JW. Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integr Cancer Ther* 2004;3:5-12.
18. Rouzaud G, Rabot S, Ratcliffe B, Duncan AJ. Influence of plant and bacterial myrosinase activity on the metabolic fate of glucosinolates in gnotobiotic rats. *Br J Nutr* 2003;90:395-404.
19. Mithen R. Glucosinolates – biochemistry, genetics, and biological activity. *Plant Growth Regul* 2001;34:91-103.
20. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 2001;56:5-51.
21. Conaway CC, Getahun SM, Liebes LL, et al. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr Cancer* 2000;38:168-178.
22. Bheemreddy RM, Jeffery EH. The metabolic fate of purified glucoraphanin in F344 rats. *J Agric Food Chem* 2007;55:2861-2866.
23. Lai RH, Keck AS, Wallig MA, et al. Evaluation of the safety and bioactivity of purified and semi-purified glucoraphanin. *Food Chem Toxicol* 2008;46:195-202.
24. Petri N, Tannergren B, Holst FA, et al. Absorption/metabolism of sulforaphane and quercetin, and regulation of phase II enzymes, in human jejunum *in vivo*. *Drug Metab Dispos* 2003;805-813.
25. Kassahun K, Davis M, Hu P, et al. Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat. Identification of phase I metabolites and glutathione conjugates. *Chem Res Toxicol* 1997;10:1228-1233.
26. Kolm RH, Danielson UH, Zhang Y, et al. Isothiocyanates as substrates for human glutathione transferases: structure-activity studies. *Biochem* 1995;311:453-459.
27. Ye AT, Kinkova-Kostova KL, Wade Y, et al. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clin Chim Acta* 2002;316:43-53.
28. Cornblatt B, Ye L, Dinkova-Kostova AT, et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis* 2007;28:1485-1490.
29. Hu R, Khor TO, Shen G, et al. Cancer chemoprevention of intestinal polyposis in apcmin/+ mice by sulforaphane, a natural product derived from cruciferous vegetable. *Carcinogenesis* 2006;27:2038-2046.
30. Shapiro TA, Fahey JW, Wade KK, et al. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. *Cancer Epidemiol Biomarkers Prev* 1998;7:1091-1100.
31. Mulcahy RT, Wartman MA, Bailey HH, Gipp JJ. Constitutive and beta-naphthalone-induced expression of the human gamma-glutamylcysteine synthetase heavy subunit gene is regulated by a distral antioxidant response element/TRE sequence. *J Biol Chem* 1997;272:7445-7454.
32. Prestera T, Talalay P, Alam J, et al. Parallel induction of heme oxygenase-1 and chemoprotective phase II enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). *Mol Med* 1995;1:827-837.
33. Brooks JD, Paton VG, Vidanes G. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. *Cancer Epidemiol Biomarkers Prev* 2001;10:949-954.
34. Basten GP, Yongping B, Williamson G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. *Carcinogenesis* 2002;23:1399-1404.
35. Thimmulappa RK, Mai KH, Srisuma S, et al. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* 2002;62:5196-5203.
36. Li W, Kong AN. Molecular mechanisms of Nrf2-mediated antioxidant response. *Mol Carcinog* 2009;48:91-104.
37. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* 2009;284:13291-13295.
38. Murashima M, Watanabe S, Zhuo XG, et al. Phase 1 study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. *Biofactors* 2004;22:271-275.
39. Han JM, Lee YJ, Lee SY, et al. Protective effect of sulforaphane against dopaminergic cell death. *J Pharmacol Exp Ther* 2007;321:249-256.
40. Kim HJ, Barajas B, Wang M, Nel AE. Nrf2 activation by sulforaphane restores the age-related decrease of TH1 immunity: role of dendritic cells. *J Allergy Clin Immunol* 2008;121:1255-1261.
41. Gao X, Kinkova-Kostova AT, Talalay P. Powerful and prolonged protection of human retinal pigment epithelial cells, keratinocytes, and mouse leukemia cells against oxidative damage: the indirect antioxidant effects of sulforaphane. *Proc Natl Acad Sci* 2001;98:15221-15226.
42. Maheo K, Morel F, Langouet S, et al. Inhibition of cytochromes p-450 and induction of glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. *Cancer Res* 1997;57:3649-3652.

43. Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett* 2008;269:291-304.
44. Parnaud G, Li P, Cassar G, et al. Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. *Nutr Cancer* 2004;48:198-206.
45. Gamet-Payrastre L, Li P, Lumeau S, et al. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in ht29 human colon cancer cells. *Cancer Res* 2000;60:1426-1433.
46. Singh AV, Xiao D, Lew KL, et al. Sulforaphane induces caspase-mediated apoptosis in cultured PC-3 human prostate cancer cells and retards growth of PC-3 xenografts *in vivo*. *Carcinogenesis* 2004;25:83-90.
47. Seligson DB, Horvath S, Shi T, et al. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005;435:1262-1266.
48. Myzak MC, Karplus PA, Chung FL, Dashwood RH. A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. *Cancer Res* 2004;5767-5774.
49. Jeong WS, Kim IW, Hu R, et al. Modulatory properties of various natural chemopreventative agents on the activation of NF-kappaB signaling pathway. *Pharm Res* 2004;21:661-670.
50. Singh SV, Srivastava SK, Choi S, et al. Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. *J Biol Chem* 2005;280:19911-19924.
51. Bertl E, Bartsch H, Gerhauser C. Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. *Mol Cancer Ther* 2006;5:575-585.
52. Asakage M, Tsuno NH, Kitayama J, et al. Sulforaphane induces inhibition of human umbilical vein endothelial cells proliferation by apoptosis. *Angiogenesis* 2006;9:83-91.
53. Kong JS, Yoo SA, Kim HS, et al. Inhibition of synovial hyperplasia, rheumatoid T cell activation, and experimental arthritis in mice by sulforaphane, a naturally occurring isothiocyanate. *Arthritis Rheum* 2010;62:159-170.
54. Fimognari C, Nusse M, Cesari R, et al. Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. *Carcinogenesis* 2002;23:581-586.
55. Pham NA, Jacobberger JW, Schimmer AD, et al. The dietary isothiocyanate sulforaphane targets pathways of apoptosis, cell cycle arrest, and oxidative stress in human pancreatic cancer cells and inhibits tumor growth in severe combined immunodeficient mice. *Mol Cancer Ther* 2004;3:1239-1247.
56. Liang H, Lai B, Yuan Q. Sulforaphane induces cell-cycle arrest and apoptosis in cultured human lung adenocarcinoma LTEP-A2 cells and retards growth of STEP-A2 xenografts *in vivo*. *J Nat Prod* 2008;71:1911-1914.
57. Tang L, Zhang Y, Jobson HE, et al. Potent activation of mitochondria-mediated apoptosis and arrest in S and M phases of cancer cells by a broccoli sprout extract. *Mol Cancer Ther* 2006;5:935-944.
58. Jackson SJ, Singletary KW. Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. *Carcinogenesis* 2004;25:219-227.
59. Azarenko O, Okouneva T, Singletary KW. Suppression of microtubule dynamic instability and turnover in MCF7 breast cancer cells by sulforaphane. *Carcinogenesis* 2008;29:2360-2368.
60. Dashwood RH, Ho E. Dietary histone deacetylase inhibitors. *Semin Cancer Biol* 2007;17:363-369.
61. Gibbs A, Schwartzman J, Deng V, Alumkal J. Sulforaphane destabilizes the androgen receptor in prostate cancer cells by inactivating histone deacetylase 6. *Proc Natl Acad Sci* 2009;106:16663-16668.
62. Myzak MC, Tong P, Dashwood WM, et al. Sulforaphane retards growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med* 2007;232:227-234.
63. Singh SV, Warin R, Xiao D, et al. Sulforaphane inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice in association with increased cytotoxicity of natural killer cells. *Cancer Res* 2009;69:2117-2125.
64. Shankar S, Ganapathy S, Srivastava R. Sulforaphane enhances the therapeutic potential of TRAIL in prostate cancer orthotopic model through regulation of apoptosis, metastasis, and angiogenesis. *Clin Cancer Res* 2008;14:6855-6865.
65. Li Y, Zhang T, Korkaya H, et al. Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells. *Clin Cancer Res* 2010;16:2580-2590.
66. Kensler TW, Chen JG, Egner PA, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo Township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 2005;14:2605-2613.
67. Noyan-Ashraf MH, Wu L, Wang R, Juurlink BH. Dietary approaches to positively influence fetal determinants of adult health. *FASEB J* 2006;20:371-373.
68. Wu L, Noyan-Ashraf MH, Facci M, et al. Dietary approach to attenuate oxidative stress, hypertension, and inflammation in the cardiovascular system. *Proc Natl Acad Sci* 2004;101:7094-7099.
69. Li N, Venkatesan MI, Miguel A, et al. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. *J Immunol* 2000;165:3393-3401.
70. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784-789.

71. Watanabe T, Tada M, Nagai H, et al. *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. *Gastroenterology* 1998;115:642-648.
72. Haristoy X, Angioi-Duprez K, Duprez A, Lozniewski A. Efficacy of sulforaphane in eradicating *Helicobacter pylori* in human gastric xenografts implanted in nude mice. *Antimicrob Agents Chemo* 2003;47:3982-3984.
73. Yanaka A, Fahey JW, Fukumoto A, et al. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected mice and humans. *Cancer Prev Res* 2009;2:353-360.
74. Galan MV, Kishan AA, Silverman AL. Oral broccoli sprouts for the treatment of *Helicobacter pylori* infection: a preliminary report. *Dig Dis Sci* 2004;49:1088-1090.
75. Yanaka A. Dietary intake of sulforaphane-rich broccoli sprouts improves gastritis in *H. pylori*-infected human subjects. November 2, 2005 Abstract #3442; presentation, American Association for Cancer Research meeting.
76. Jansen PL, Mulder GJ, Burchell B, Bock KW. New developments in glucuronidation research: report of a workshop on "glucuronidation, its role in health and disease." *Hepatology* 1992;15:532-544.
77. Peterson S, Bigler J, Horner NK, et al. Cruciferae interact with UGT1A1*28 polymorphism to determine serum bilirubin levels in humans. *J Nutr* 2005;135:1051-1055.
78. Yamanishi Y, Firestein GS. Pathogenesis of rheumatoid arthritis: the role of synoviocytes. *Rheum Dis Clin North Am* 2001;27:355-371.
79. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vis* 1999;5:32.
80. Gao X, Talalay P. Induction of phase 2 genes by sulforaphane protects retinal pigment epithelial cells against photooxidative damage. *Proc Natl Acad Sci* 2004;101:10446-10451.
81. Vauzour D, Buonfiglio M, Corona G, et al. Sulforaphane protects cortical neurons against 5-S-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, Nrf-2 and the upregulation of detoxification enzymes. *Mol Nutr Food Res* 2010;54:532-542.
82. Tarozzi A, Morroni F, Merlicco A, et al. Sulforphane as an inducer of glutathione prevents oxidative stress-induced cell death in dopaminergic-like neuroblastoma cell line. *J Neurochem* 2009;111:1161-1171.
83. Han JM, Lee YJ, Lee SY, et al. Protective effect of sulforaphane against dopaminergic cell death. *J Pharmacol Exp Ther* 2007;321:249-256.