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Review

Translating knowledge generated by epidemiological and *in vitro* studies into dietary cancer prevention

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Epidemiological studies have identified an inverse relationship between ingestion of plant foods and cancer risk. However, only ~2/3 of such studies show this association. Clinical trials based on epidemiological findings require preclinical studies to provide insight into reproducibility. The beta carotene story is an example of clinical trials based on epidemiological data, before mechanism, dose or the bioactive component had been clearly identified. Results showed rather than prevention, an increase in lung cancer in smokers. Epidemiological studies are used successfully to generate hypotheses for *in vitro* mechanistic studies of isolated components from plant foods, such as sulforaphane from broccoli. Yet even these studies are insufficient to plan clinical trials of whole foods, since bioavailability, disposition, dose, and effects of the food matrix remain unknown. Evidence-based information, from animal and small clinical studies carried out prior to clinical trials can assure an optimal design. Research into effects of broccoli and sulforaphane make an excellent example of how data gaps have closed between epidemiology and clinical trials. Data on efficacy of broccoli in animal cancer prevention studies are strong, and small clinical studies are emerging. The time is right for clinical trials of purified and semipurified sulforaphane, as well as whole broccoli.

Keywords: Broccoli / Cruciferous vegetables / Dietary cancer prevention / Epidemiology / Sulforaphane

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1 Introduction

A growing number of scientists are studying dietary cancer prevention, yet often data are missing on how to translate much of the research generated into clear guidelines for the consumer. For example, "broccoli may decrease risk for prostate cancer" sounds clear, but leaves both the consumer and the clinician interested in designing a robust clinical trial unsure of dose, frequency of inclusion into the diet, or whether variety or preparation method is important for gaining the health benefit, or even if a sulforaphane (SF) supplement could replace whole broccoli. This lack of detail is very different from guidelines for use of drugs, where directions about frequency, dose, contra-indications, and potential adverse side effects to watch for are clearly outlined by the manufacturer on the package insert. Also, generic alternatives are carefully evaluated for equivalency. As a result of this lack of information on foods that have health benefits, neither the clinician nor the consumer has any knowledge of an effective dose. This is of particular concern when considering cancer prevention, since there is no easy, short-term endpoint/health outcome in order to judge effectiveness, such as plasma cholesterol levels for cardiovascular health. Clinical trials carried out prior to filling these knowledge gaps may not be optimized for these parameters and may provide confusing, disappointing, and maybe even harmful results [1].

If diet is to play a meaningful role in cancer prevention in the US, clear guidelines, based on consistent clinical trials must be developed for the consumer. Substantial data gaps must be filled, to provide the detailed, evidence-based information necessary for the optimized design of these clinical trials:

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Abbreviations: ESP, epithiospecifier protein; **PhIP,** 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; **RR,** relative risk; **SF,** sulforaphane

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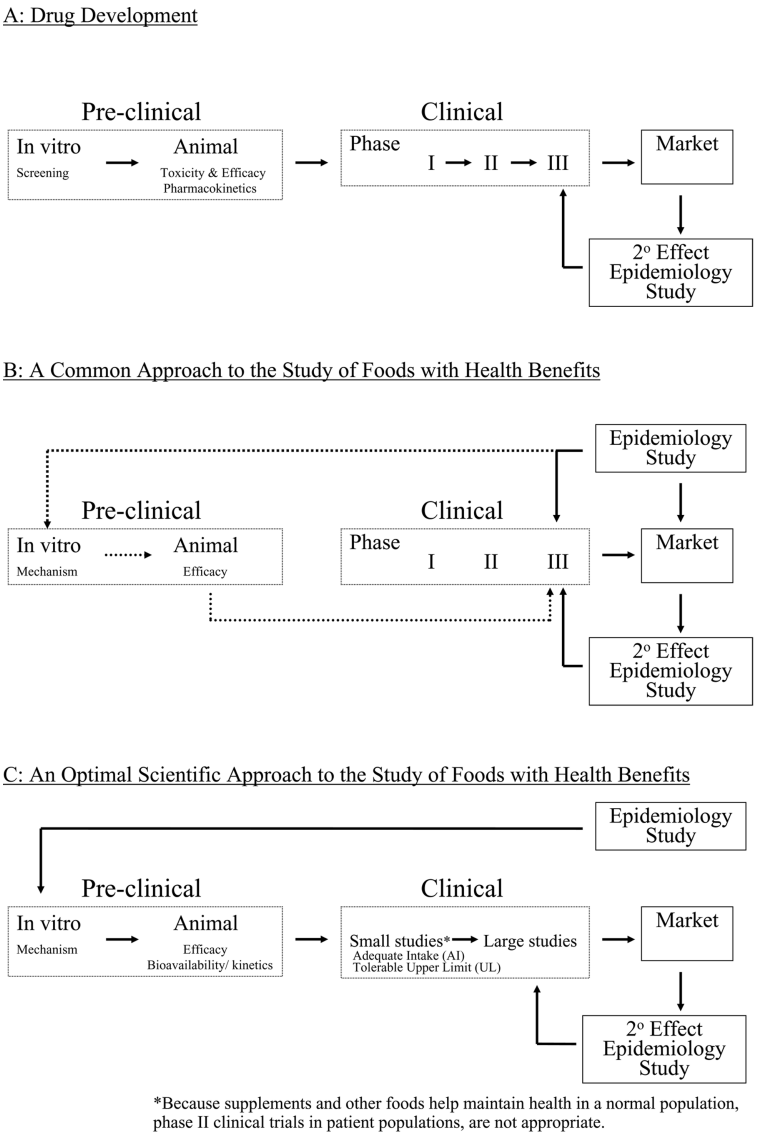

Figure 1. Drug development (A), a common approach to the study of foods with health benefits (B), and an optimal scientific approach to the study of foods with health benefits (C). A solid line represent common practice and a dotted line represent less frequent occurrence.

(i) Epidemiological data alone cannot provide the information necessary to design a robust clinical trial.

(ii) *In vitro* data, even based on epidemiological studies, cannot provide the necessary detail or justification for designing a robust clinical trial.

(iii) Animal modeling of efficacy, bioavailability, and kinetics are essential for designing a robust clinical trial.

This paper will discuss these issues and use research on broccoli and cancer prevention as a working example, where scientists are aiming to close these data gaps.

Methods for the evaluation of safety and efficacy of drugs undergoing development typically start with *in vitro* screening assays. All steps are well established, as outlined in Fig 1A. In contrast, identification of health benefits of plant foods more typically begins with an epidemiological study, possibly based on historical use of a food for health maintenance, and either moves directly to marketing or to a clinical trial based on the epidemiological data, Fig 1B. Sometimes this confirms the hypothesized benefit, as with cranberry juice [2], but at other times, because there are no data on dose, bioavailability or kinetics, the study may not be optimized to show the expected benefit. Clinical trials are expensive in time and money. Also, once a trial fails to show the proposed health benefit, the impact of this information on consumers and physicians is so great that it can take years and more than one robust clinical trial to correct. An early example of the negative impact of a clinical study without optimal design was the "bursting of the oat bran bubble" – where healthy young nurses were used to evaluate whether oat bran could lower cholesterol, and the oat bran diet was found to have no effect [3]. Today we know that bioactive foods often normalize parameters such as

Table 1. Dietary crucifer intake associated with lowered cancer risk

Cancer site	Intake	Risk	*p*-value
Bladder [10]	>5 Serving/week	RR 0.49	0.008
Lung [60]	= 0.5 Serving/day	OR 0.31	CI (0.1 – 0.92)
Lymphoma [70]	>5 Serving/week	RR 0.67	0.03
Prostate [12]	>3 Serving/week	OR 0.59	0.02
Prostate [71]	=5 Serving/week	OR 0.61	0.006
Breast [72]	Quartile 4	OR 0.50	0.01
Kidney [73]	Quartile 4	OR 0.53	0.001
Ovarian [74]	>0.83 Serving/day	HR 0.75	0.03

OR, odds ratio; HR, hazard ratio; RR, relative risk; CI confidence interval.

cholesterol [4, 5], and thus would be seen to have little effect on these young women with normal-range cholesterol levels. But at the time, the trial results were interpreted to mean that oat bran cannot lower plasma cholesterol, regardless of the starting level. Preclinical studies, followed by small clinical studies, can provide much needed data for development of a full randomized placebo control clinical trial, Fig 1C.

2 Epidemiological data alone are not sufficient to design a clinical trial

Evidence-based medicine cautions the use of epidemiological studies alone to set policy for therapeutic care [6]. Whereas epidemiological studies are an excellent source of material for hypothesis generation, basing marketing, or lifestyle guidelines on epidemiological data alone may not always prove useful. A recent report even suggested that epidemiological studies are considerably less than 50% reproducible [7]. This low reproducibility might be due to our lack of knowledge of different aspects of the food under study, such as changes in the content of bioactive components with plant variety or cooking method. Of all the epidemiological studies reviewed in the WCRF/AICR report in 1997, only 80% of vegetable studies and 69% of the cruciferous vegetable studies showed significant protection [8]. The rest of the studies showed no effect or even adverse effects. Evidence-based medicine has grown to be synonymous with the ranking of clinical data into categories based on their freedom from bias. Thus the strongest evidence for clinical practice – and by extension for dietary guidelines for health and wellness – comes from double blind placebo-controlled trials involving a homogeneous population, and in the case of foods for wellness, involving individuals in good health. By contrast, epidemiological studies rank low, with substantial potential for bias.

Epidemiological studies suggest that cruciferous vegetables, including broccoli, may provide more protection than fruits and vegetables in general, against many different cancers [9], making them excellent candidates for dietary cancer prevention if clear direction for use is given to the consumer. Multiple studies suggest that 3 – 5 servings a week can provide significant protection (Table 1). For example, in the Health Professionals Follow-Up Study that monitored 47 909 men over 10 years and reported 252 cases of bladder cancer, analysis of food frequency questionnaires collected thrice revealed a significant 51% reduction in risk for bladder cancer (relative risk, RR 0.49, $p = 0.008$) in individuals ingesting five or more servings of cruciferous vegetables a week, compared to those ingesting one or fewer servings/week (Table 1 [10]). Consumption of green leafy vegetables (RR = 0.99, 0.63 – 1.56, $p = 0.81$) or all fruit and vegetables (RR = 0.72, CI 0.47 – 1.09, $p = 0.09$) did not show a significant risk reduction [10]. Interestingly, in many studies comparing all fruit and vegetable intake with incidence of cancer, cancer risk is lowered by 20 – 40% in the highest intake group, compared to the lowest [11], whereas separating out the data for consumption of crucifers, those eating 3 – 5 servings of crucifers a week (the highest US intake group), are often associated with a 50%, or even 60% decrease in risk compared to those who eat fewer than one serving of crucifers *per* week [10].

However, not all studies show a decrease in cancer risk with increased crucifer intake. In a review of 87 case control studies, although 58 showed an inverse association between consumption of cruciferous vegetables and cancer risk, only 39 of these showed a significant inverse risk [9]. Of the seven cohort studies reviewed in the same article, only five studies showed a significant decrease in risk with increased crucifer consumption. This lack of reproducibility points to the need for additional types of studies, in order to understand and control variables. If a clinical trial were to be performed based solely on the epidemiological studies, the design might not be optimized correctly and might fail to correctly test the efficacy of the broccoli or other crucifer under study. For example, in two large studies evaluating risk for prostate cancer in men consuming cruciferous vegetables, one reported that 3 – 5 servings of crucifers a week compared to 1 serving or less lowers risk for prostate cancer by 41% (95%CI 0.39 – 0.90, $p = 0.02$; Table 1) [12]. The other found that the effect of crucifers was not significant except for a sub-set of men with early-stage cancer [13]. Furthermore, early prostate cancer screening may be a con-

Table 2. Cancer-protective mechanisms reported for SF in cell culture studies

Endpoints	Effect	SF dose	Cancer cell type	References
Induce phase II	↑ 2-Fold (24 h)	0.4 – 0.8 μM	Liver (mouse, Hepa 1c1c7)	[30]
Detoxification	↑ 1.5 – 2-Fold (48 h)	2 – 5 μM	Liver (human, HepG2)	[75]
	↑ 1.3 – 1.6-Fold (24 h)	5 – 10 μM	Colon (human, Caco-2)	[75]
	↑ 1.1 – 2.1-Fold (24 h)	0.5 – 5 μM	Prostate (human, LNCaP, MDA PCa2b)	[76]
Inhibit histone	↓ 30 – 40%	15 μM	Prostate (human, BPH-1, LNCaP, PC-3)	[28]
Deacetylation	↓ 20 – 65%	5 – 25 μM	Breast (human, MCF-7, T47D, MDA-MB-231, and 468)	[77]
Cell cycle arrest	G2/M arrest (24 – 72 h)	15 μM	Colon (human, HT29)	[50]
	G2/M arrest (24 h)	25 – 50 μM	Colon (human, Caco-2)	[78]
	G2/M arrest (24 h)	10 – 40 μM	Prostate (human, LNCaP)	[79]
	G2/M arrest (24 h)	15 μM	Breast (human, MCF-7)	[52]
	G2/M arrest (48 h)	30 μM	T-cells (human, Junkat)	[80]
Apoptosis	2 – 5-Fold (24 – 72 h)	15 μM	Colon (human, HT29)	[50]
	5 – 10-Fold (24 h)	10-20 μM	Prostate (human PC3, LNCaP)	[81, 79]
	8 – 16-Fold (24 – 48 h)	30 μM	T-cells (human, Junkat)	[80]
Inhibit cell	↓ 50%	27 μM	Bladder (human, T24)	[82]
Growth	↓ 50%	40 μM	Ovarian (human, SKOV3)	[83]
	↓ 50%	25 μM	Ovarian (mouse, C3, T3)	
	↓ 50%	8-10 μM	Breast (human, MCF-7, T47D, MDA-MB-231, and 468)	[77]
	↓ 75%	15 μM	Breast (human, MCF-7)	[52]
	↓ 50 – 60%	10-30 μM	T-cells (human, Junkat)	[80]
	↓ 50%	12 μM	Bladder (human, RT4)	[84]

founding influence, since only men who are screened for prostate cancer are identified during early stage disease [14].

Perhaps the best justification for using epidemiological data as hypothesis-generating but not for determining efficacy in place of preclinical studies comes from the study of the dietary supplement, beta carotene. In dietary intake studies, carotenoids can be used as a biochemical measure to check reported fruit and vegetable intake. In many such epidemiological studies, serum beta carotene was found to be inversely related to cancer incidence, suggesting that a diet rich in fruits and vegetables and/or beta carotene is associated with a lower risk for a number of cancers [15]. In addition, not only was serum beta carotene found to be lower in smokers than in nonsmokers, but the Linxian Nutrition Intervention Trial found that total cancer mortality was decreased by 13% in those given selenium, beta-carotene, and vitamin E [16]. However, two large clinical trials then found that beta-carotene supplements enhanced, rather than prevented, the incidence of lung cancer in smokers. Specifically, the alpha-tocopherol/beta carotene (ATBC) study, a US/Finland joint study of dietary supplementation and lung cancer prevention, found that in 59 – 65 year olds, beta carotene increased lung cancer risk by 16%, almost overshadowing a secondary finding that vitamin E supplementation was associated with a >30% decrease in prostate cancer incidence [1]. In the beta-carotene and retinol efficacy trial (CARET), a combination of beta-carotene (30 mg) and retinyl palmitate (25 000 IU vitamin A) was given daily to 18 314 men and women at high risk for lung cancer [17]. Due to increased lung cancer incidence (36%) and mortality (59%) in the combination arm compared to the placebo arm, the trial was terminated 21 months early.

Since the early 90's, when fruits and vegetables were considered beneficial possibly because of their carotenoid content, numerous studies using cell cultures and animals have helped to persuade scientists that plant foods contain secondary metabolites with health-promoting activity [18]. Such studies have helped bring the science to the point where there is sufficient significant scientific agreement that individuals with a high intake of fruits and vegetables have a lower risk for developing a number of cancers, that the Food and Drug Administration has approved a health claim to this effect (http://vm.cfsan.fda.gov/~dms/flg-6c.html). Whether this benefit truly stretches across all fruits and vegetables, or whether the claim should be limited to a select number of plant foods for which there are strong data, has not been addressed. A very recent clinical trial, on diet and recurrence of breast cancer, evaluated the effect of a high fruit and vegetable diet, in addition to high fiber and low fat, and found no effect of fruits and vegetables [19]. Yet a quick review of the study design shows that the women were taking in 6 – 8 servings of fruits and vegetables before the study started – and the control group continued this diet, while the experimental arm improved their diet to 10 – 12 servings of fruits and vegetables [19]. This design is not supported by epidemiological data, which show differences between very low intake (one or fewer servings/day) and an intake resembling five-a-day [20, 21]. Furthermore, average intake in the US is 2 – 3 servings [22]. Unfortunately, not recognizing the potentially confounding effect of the very high control intake, the Womens' Healthy Eating and

Living (WHEL) study concluded that fruits and vegetables are without benefit in regard to recurrence of breast cancer. Such a message is both more than the study can say and highly confusing to the consumer.

3 An important role for *in vitro* studies is to determine mechanism

Mechanistic evidence for bioactivity is often derived from *in vitro* studies of purified components isolated from foods, and has gone far to persuade scientists of the potential benefit of plant foods, even though cell culture studies do not address disposition. For example, a large body of literature shows that isothiocyanates added to cell culture are able to upregulate the phase II detoxification enzymes glutathione S-transferases and quinone reductase, supporting the suggestion that *in vivo* they might enhance clearance of chemical carcinogens and thus block chemical carcinogenesis [23]. Further *in vitro* studies revealed an Nrf2-dependent mechanism for the upregulation of genes bearing an antioxidant response element (ARE) in the promoter region. Isothiocyanates cause translocation of Nrf2 into the nucleus to bind to the ARE. The exact manner by which isothiocyanates trigger Nrf2 translocation is still undetermined [24]. Yet cell culture studies have also revealed a role for isothiocyanates in pathways other than blocking carcinogenesis. *In vitro* studies show that isothiocyanates can disrupt growth of cancer cells, specifically arresting the cell cycle, slowing or stopping proliferation, enhancing apoptosis, restoring silenced glutathione-S-transferase P1 activity, and inhibiting histone deacetylase activity [25–28]. It is not yet clear if any of the enzymes involved in these reactions are Nrf2-dependent and/or require higher concentrations than the upregulation of detoxification enzymes [29]. The effects of bioactive components on cellular physiology may change with both dose and cell type (Table 2). Thus very low concentrations of the isothiocyanate SF, in the 0.4–0.8 μM range, are able to trigger a number of Nrf2-dependent changes in detoxification enzyme levels [30]. At somewhat greater levels, the cell cycle is inhibited, cell growth is inhibited, and apoptosis is seen to occur. Whether the concentrations necessary to cause these changes in cultured cells reflect the necessary plasma levels for such activities to occur *in vivo* is not known. Maximum plasma levels of SF were only 7.3 μM, even when subjects drank a soup of a hybrid broccoli with three times the typical level of glucosinolates [31], bringing into question whether some cell studies reflect *in vivo* mechanisms when they use up to 100 μM SF (Table 2). Upregulation of the apoptosis pathway has been reported in animals following administration of Brussels sprouts juice [32] and SF (600 ppm in the diet [33]), suggesting that apoptosis occurs at lower concentrations *in vivo* than *in vitro*, or possibly that SF concentrates in some cell types [34]. Alternatively, the active form of the agent, and the potency, may differ between *in vivo* and *in vitro*. For example, one possible bioactive form *in vivo* might be the SF cysteine conjugate, which was detected in relatively high concentration (0.8 μM) in human plasma after consumption of a broccoli soup containing 100 g broccoli [35]. Growth of cancer cells is adversely affected at lower isothiocyanate levels than is growth of nontransformed cells [36, 37] or detransformed cells [38]. It remains to be determined what the margin of safety is between causation of apoptosis in cancer cells and normal cells *in vivo*. These data remind us that all compounds are toxic and that a safe and tolerable upper level, typically referred to as "UL" (Fig. 1C) needs to be determined, particularly when bioactive components are isolated from whole foods and provided as dietary supplements. Toxicity is commonly evaluated in cell culture by cell kill, but there may be a number of responses to a bioactive agent that, under certain conditions, are not of benefit. For example, by altering detoxification enzymes, bioactive components may alter hormone metabolism, something rarely evaluated in cell culture. Some products of broccoli and of other bioactive foods such as garlic can increase clotting time [39]. Similarly, SF appears to trigger multidrug resistance, a useful system for ridding the cell of foreign compounds except when one is undergoing drug therapy [40]. Potential interactions with drugs, dietary supplements and other bioactive food components, positive or negative, may also be of concern, such as vitamin K-rich Brussels sprouts and warfarin [41] or St. John's wort and drugs undergoing metabolism through CYP3A [42].

Many foods contain more than one bioactive component, and yet scientists have a tendency to identify a major bioactive and then equate effects of that individual component with the effect of the whole food. An example is the highly active and well-studied isothiocyanate SF [30]. Much of the cell culture literature on mechanism infers that SF is responsible for the bioactivity of dietary broccoli, whereas there are other bioactives present in the whole food. One major additional bioactive component is indole-3-carbinol, the hydrolysis product of glucobrassicin [43]. Indole-3-carbinol is bioactivated to a number of highly active acid condensation products as it passes through the acid stomach, which process is difficult to model in cell culture. These metabolites act very differently from SF, able to trigger the aryl hydrocarbon receptor and cause upregulation of CYP1A through interaction of the receptor complex with a specific gene sequence in the promoter region, the xenobiotic response element. Finding that components in broccoli upregulate CYP1A, considered to activate dietary carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), is dichotomous given that both SF and whole broccoli are associated with anticarcinogenic properties. This little-studied area is very much in need of work. One elegant clinical study evaluated urinary PhIP and found that consumption of 250 g of Brussels sprouts and broccoli

Table 3. Efficacy of broccoli, broccoli sprouts and SF in animal studies of cancer prevention

Cancer	Animal model	Main protective effect	Amount	Duration	Reference
Prostate	Dunning rat	42% Less growth 52% Less growth	10% Broccoli 10% Broccoli and 10% tomato combo	18 wk	[85]
Prostate	PC-3 Mice Xen.	40% Less growth	443 ppm SF	21 days	[28]
Small Intestine	ApcMin/+ mice	25% Less multiplicity 47% Less multiplicity	300 ppm SF 600 ppm SF	3 wk	[86]
Skin	SKH-1 mice (UV-trt)	50% Less incidence and multiplicity	1 μmol SF/mouse as broccoli sprout extract (topical)	11 wk	[87]
Skin	CD-1 mice (DMBA-trt)	61% Less incidence and 81% multiplicity 89% Less incidence and 96% multiplicity 72% Less incidence and 98% multiplicity	1 μmol SF/mouse (topical) 5 μmol SF/mouse (topical) 10 μmol SF/mouse (topical)	15 wk	[49]
Mammary	F3II BALB/C mice Xen.	60% Less growth	15 nmol SF/mouse (i.v.)	13 days	[52]
Mammary	SD rats (DMBA trt)	45% Less incidence and 63% multiplicity 66% Less incidence and 69% multiplicity 89% Less incidence and 91% multiplicity 89% Less incidence and 75% multiplicity 66% Less incidence and 75% multiplicity	25 μmol Isothiocyanates (gavage)[a)] 50 μmol Isothiocyanates (gavage)[a)] 100 μmol Isothiocyanates (gavage)[a)] 25 μmol Glucosinolates (gavage)[a)] 100 μmol Glucosinolates (gavage)[a)]	150 days	[88]

a) Given as a 3-day-old broccoli sprout extract.

for 12 days significantly increased the urinary PhIP excretion hence lowering the amount of PhIP retained in the body [44]. A recent study evaluated the interactive effect of SF and a major indole-3-carbinol metabolite di-indolyl methane [45]. They found that whether the combination was antagonistic or synergistic depended upon the concentration, showing the need for the study of whole food extracts rather than single components.

Cell culture studies can be very informative about mechanism, but it is not enough to know that a component has bioactivity in cell culture. Cell culture studies may use doses that cannot be achieved physiologically, and they cannot provide information on bioavailability. Additionally, they may miss interactions with additional components in the whole food. For these reasons, *in vitro* studies do not extrapolate directly to dietary effects. The greatest gap in our understanding of health effects of bioactive food components may be details on disposition: bioavailability from different products, distribution, and metabolism, the effective dose and the tolerable upper level. Animal studies can provide information on many of these questions to permit moving forward to small clinical studies.

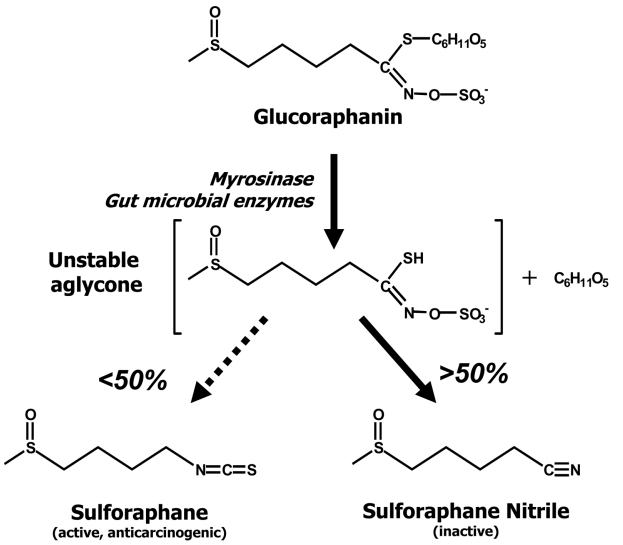

Figure 2. Hydrolysis of glucoraphanin to SF and SF nitrile.

4 Animal studies for determining efficacy, bioavailability, and kinetics

Animal studies can compare the purified component(s) used in cell culture with the complex foods in our diet, confirming (or refuting) the mechanisms identified in cell culture. Although animal studies do not always reflect *in vitro* findings, it is imperative to compare similar doses, forms, and extent of exposure before rejecting *in vitro* findings. Animal studies can highlight or dismiss concerns over bioavailability, efficacy, and kinetics. Frequently only a fraction of a dietary dose is absorbed. Animal studies can provide information about digestion, kinetics, and metabolism that may, for example, suggest specific processing methods

to optimize bioavailability. Once a metabolic pathway is identified in animals, detecting and confirming it in a small clinical study can be relatively straightforward.

Effective cancer prevention by bioactive foods can be evaluated directly in animal feeding studies, as shown for studies involving broccoli, a broccoli sprouts extract, or the purified components glucoraphanin or SF (Table 3). These studies can be used to relate urinary and plasma levels of a bioactive component to changes in biomarkers of efficacy. Animal studies can also identify toxicities associated with high doses, providing signals for potential adverse events in future clinical studies. Very high crucifer consumption has been associated with changes in thyroid hormones in animals [46], but this was not seen when a group of subjects were given broccoli sprouts extract [47].

Animal studies, like cell culture studies, have long confirmed that SF and other isothiocyanates can modify detoxification enzymes, increasing phase II detoxification, and inhibiting phase I carcinogen activation, and lower DNA adduct formation. But unlike cell culture, animal studies also show increased clearance of carcinogens from the body, confirming an anti-initiation mechanism [48]. Once considered limited to anti-initiation, more recently animal studies have confirmed suggestions from cell culture studies that SF can inhibit all stages of the carcinogenic process [49 – 52]. In fact, SF may be more effective against promotion than initiation [49]. In light of increasing evidence for an antitumorigenic role for SF, it becomes even more important to understand SF availability from whole foods, to offer the general public this mechanism for decreasing risk.

Bioactive isothiocyanates such as SF are present in cruciferous vegetables as thioglucoside precursors, glucosinolates, which must undergo hydrolysis to release isothiocyanates (Fig. 2). Human and other animal tissues contain no thiohydrolase activity, and addition of glucosinolates to cells in culture has no effect [53]. However, both cruciferous plants and microbiota in the gut can hydrolyze glucosinolates, releasing active isothiocyanates. When the plant thiohydrolase, myrosinase, is added together with glucosinolates into cell culture, isothiocyanates are released and activity is seen [53]. In plant tissue, glucosinolates are spatially separated from myrosinase. Thus glucosinolates remain chemically stable until the plant tissue is disrupted due to food preparation or chewing. When glucosinolates come into contact with myrosinase, the *S*-glucose bond is cleaved, leaving an unstable aglycone intermediate, which rapidly rearranges nonenzymatically to form an isothiocyanate and/or one of several alternative products. We have shown that glucoraphanin is hydrolyzed in part to SF, in part to an inactive nitrile (Fig. 2) [54, 55]. For some time it has been known that in plant tissue nitrile formation is favored by low pH and free iron [56]. Recently we identified in broccoli a myrosinase cofactor, the epithiospecifier protein (ESP), and showed that it supports nitrile formation at the expense of SF [57]. The importance of this is that SF nitrile lacks bioactivity both in cell culture and in animals [54], so when nitrile formation occurs at the expense of SF formation, this results in lessening of the health benefit. Choosing a broccoli variety low in ESP, or destroying ESP through processing may greatly enhance bioactivity. Unfortunately, preliminary data suggest that nitrile formation may also be supported by microflora in the gut [58].

5 Methods developed in animals can be applied successfully in small clinical studies

Once efficacy has been established in animal models, translation to humans requires measurement of exposure and disposition, as well as measurement of efficacy. Whereas animal studies do not always appear to translate successfully to clinical findings, this is often because of differences in dose, exposure route, duration and frequency of exposure, or genetic diversity within the human population under study [59, 60]. Biomarkers of exposure that were developed in animals, such as measurement of urinary SF conjugates and plasma SF, have been utilized successfully in small human clinical trials using broccoli and broccoli preparations [47, 61] (Table 4). SF is metabolized to SF mercapturate (*N*-acetyl cysteine conjugate) in animals [62]. These same metabolites are found in human urine. These metabolites appear to be a better measure of systemic exposure to SF than dietary intake, in most part because fractional uptake varies considerably based on the food preparation. Preformed SF in a broccoli sprout matrix appears to be highly bioavailable, with up to 80% of dietary intake appearing in the urine [61]. In contrast, recovery from a sprouts preparation with inactivated myrosinase was only 12%. Fresh sprouts chewed to allow hydrolysis in the mouth provided an intermediate recovery of 42%. Similarly, studies of urinary recovery from ingested fresh broccoli and steamed broccoli suggest that steamed broccoli provides a recovery of only 12%, and fresh broccoli, which has the active myrosinase at the time of ingestion, provides an improved recovery of ~30% [63]. Given that hydrolysis in the absence of an active myrosinase depends upon colonic microbiota, these data suggest either that hydrolysis by colonic microbiota is mostly to a product other than SF, or that glucoraphanin is mostly absorbed and/or degraded prior to reaching the colon.

Relatively few studies have evaluated plasma SF in animals or humans. When adult male F344 rats were provided a single oral dose of 50 μmol SF (~350 μmol/kg rat), plasma SF levels peaked around 20 μM at 4 h after dosing [64]. The half life was reported as 2.2 h. However, this dose is substantially greater than typically ingested by man. In a small clinical study, subjects consumed ~200 g fresh broccoli (reported as 0.48 μmol SF/g fresh weight, which is

Table 4. Small clinical studies of SF bioavailability and bioactivity using broccoli and broccoli sprouts

Preparation	Amount	Duration	Design	Effects	Reference
Broccoli sprouts	68 g (593 μmol SF)	1 meal	18–55 years (n = 3)	HDAC activity in PBMC inhibited 3 and 6 h postmeal	[28]
Broccoli	500 g	6 days	5 men and 5 women	Liver CYP1A2 and 2A6 up-regulated	[69]
Broccoli sprout	200 μmol SF	1 meal	8 women	1 h postmeal: breast tissue contained 1.5–2 pmol/mg, plasma 0.9 μM and urine 159 μM	[47]
Broccoli sprouts	25 μmol ITC 25 μmol GS	3x/day for 7 days	11 men and 1 woman	Urinary recovery: 70% of the ITC, 20% of the GS as ITC. No adverse effect on thyroid or liver enzymes compared to placebo	[47] [89]
Broccoli sprouts	200 μmol ITC	1 meal	4 men	Peak plasma, serum, and RBC (ITC): 0.9–2.3 μM at 1 h postmeal. $t_{1/2}$ = 1.8 ± 0.1 h Urinary recovery: 58% after 8 h	[90]
Broccoli	200 g fresh (136 μmol GP) 200 g steamed (94 μmol GP)	1 meal	12 men	Urinary recovery: 19% recovery as SFM Urinary recovery: 7% recovery as SFM	[63]
Broccoli sprouts	111 μmol ITC	1 meal	43–53 years, cross over, n = 4	Urinary recovery: 80% recovery of ITC	[61]
Broccoli sprouts	111 μmol GS 12 g (100 μmol GS)	1 meal	25–75 years, 3–11 subjects	Urinary recovery: 12% recovery as ITC Urinary recovery: 42% for chewed *vs.* 26% for whole sprouts	[61]
Broccoli	500 g	12 days	n = 18	CYP1A2 increased 19% 2/16alpha-hydroxyestrone ratio increased 29.5%	[68]

GP, glucoraphanin; GS, glucosinolates; HDAC, histone deacetylase; ITC, isothiocyanates; PBMC, peripheral blood mononuclear cells; RBC, red blood cells; SF, sulforaphane; SFM, sulforaphane mercapturic acid.

roughly 1.5 μmol SF/kg man); which resulted in elevated plasma levels for 0–10 h postingestion (range 0.8–1.7 μM isothiocyanate equivalents) compared to a premeal plasma level of 0.6–1.1 μM isothiocyanate equivalents; no statistical analysis was reported [63]. In a more recent study using MS/MS to enhance sensitivity, when subjects consumed 100 g broccoli as a soup, this gave peak free SF plasma levels of 0.65 μM, and all forms of SF (free and conjugated) ~2 μM [35]. Particularly in light of the effective doses for different bioactivities in cell culture (Table 2), it will be interesting in the future to determine if the low plasma levels of SF typical of dietary sources will provide selectivity of effects and/or cell types. A recent small clinical study found that 1 h after drinking a broccoli sprouts extract, normal breast tissue contained 1.5–2.0 pmol SF/mg, similar to plasma levels, which were 0.9 μM [47].

Availability of robust biomarkers of efficacy in healthy individuals is still extremely sparse. Whereas multiple biomarkers for effective maintenance of health have been developed in association with cardiac health, there remains a lack of biomarkers for determining successful prevention of cancers. As these are identified, it will be necessary to determine how new biomarkers can best be utilized to help the general public choose a diet that prevents cancer. One example of a biomarker that may indicate an effective dose of the broccoli component indole-3-carbinol is a change in the urinary 2-hydroxy/16alpha-hydroxy estrone ratio, shown in animal studies to correlate with a decrease in chemically induced mammary cancer [65]. When indole-3-carbinol was given to women, a similar change was seen in the urinary estrogen metabolites [66]. Furthermore, comparing women who have been diagnosed with breast cancer and those that have not, there was a significant difference in their urinary estradiol metabolite ratio [67]. One human study giving 500 g broccoli daily for 12 days reported a significant increase (29.5%) in 2-hydroxy/16alpha-hydroxy estrone ratio [68] and they also found a significant increase in CYP 1A2 activity which is considered the enzyme responsible for increased production of 2-hydroxy estrone [68]. A recent report used caffeine metabolism to confirm that CYP1A2 is elevated after a broccoli meal [69]. Another potential efficacy biomarker was recently reported. Three individuals were given 68 g broccoli sprouts and inhibition of histone deacetylase was seen 3 and 6 h after the meal. This was a confirmation of findings in mice, and in cultured cells treated with SF [28]. The extent to which acute inhibition of histone deacetylase activity plays a causative role in cancer prevention remains to be determined.

In summary, it is essential to conduct detailed preclinical studies and small human studies before taking the step to

fully randomized, double blind placebo-controlled human trials. Furthermore, data on efficacy of broccoli in animal cancer prevention studies are strong, and small clinical studies are emerging that show bioavailability, disposition, and biomarkers. The time is right for clinical trials of purified and semipurified SF, as well as whole broccoli.

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6 References

[1] Albanes, D., Heinonen, O. P., Huttunen, J. K., Taylor, P. R., *et al*., Effects of alpha-tocopherol and beta-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. *Am. J. Clin. Nutr.* 1995, *62*, 1427S – 1430S.

[2] Avorn, J., Monane, M., Gurwitz, J. H., Glynn, R. J., *et al*., Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *JAMA* 1994, *271*, 751 – 754.

[3] Swain, J. F., Rouse, I. L., Curley, C. B., Sacks, F. M., Comparison of the effects of oat bran and low-fiber wheat on serum lipoprotein levels and blood pressure. *N. Engl. J. Med.* 1990, *322*, 147 – 152.

[4] Reyna-Villasmil, N., Bermudez-Pirela, V., Mengual-Moreno, E., Arias, N., *et al*., Oat-derived beta-glucan significantly improves HDLC and diminishes LDLC and non-HDL cholesterol in overweight individuals with mild hypercholesterolemia. *Am. J. Ther.* 2007, *14*, 203 – 212.

[5] Karmally, W., Montez, M. G., Palmas, W., Martinez, W., *et al*., Cholesterol-lowering benefits of oat-containing cereal in Hispanic americans. *J. Am. Diet Assoc*. 2005, *105*, 967 – 970.

[6] Harlan, W. R., Jr., Research on complementary and alternative medicine using randomized controlled trials. *J. Altern. Complement. Med.* 2001, *7*, S45 – S52.

[7] Tuma, R. S., Statisticians set sights on observational studies. *J. Natl. Cancer Inst.* 2007, *99*, 664 – 665, 668.

[8] Potter, J. D. (Ed.), in: *Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective*, World Cancer Research Fund and American Institute for Cancer Research, Washington, DC 1997, p. 442.

[9] Verhoeven, D. T., Goldbohm, R. A., van Poppel, G., Verhagen, H., *et al*., Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 1996, *5*, 733 – 748.

[10] Michaud, D. S., Spiegelman, D., Clinton, S. K., Rimm, E. B., *et al*., Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *J. Natl. Cancer Inst.* 1999, *91*, 605 – 613.

[11] Riboli, E., Norat, T., Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am. J. Clin. Nutr.* 2003, *78*, 559S – 569S.

[12] Cohen, J. H., Kristal, A. R., Stanford, J. L., Fruit and vegetable intakes and prostate cancer risk. *J. Natl. Cancer Inst.* 2000, *92*, 61 – 68.

[13] Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J., *et al*., A prospective study of cruciferous vegetables and prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* 2003, *12*, 1403 – 1409.

[14] Kristal, A. R., Stanford, J. L., Cruciferous vegetables and prostate cancer risk: Confounding by PSA screening. *Cancer Epidemiol. Biomarkers Prev.* 2004, *13*, 1265.

[15] van Poppel, G., Goldbohm, R. A., Epidemiologic evidence for beta-carotene and cancer prevention. *Am. J. Clin. Nutr.* 1995, *62*, 1393S – 1402S.

[16] Blot, W. J., Li, J. Y., Taylor, P. R., Guo, W., *et al*., Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J. Natl. Cancer Inst.* 1993, *85*, 1483 – 1492.

[17] Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., *et al*., Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J. Natl. Cancer Inst.* 1996, *88*, 1550 – 1559.

[18] Surh, Y. J., Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer* 2003, *3*, 768 – 780.

[19] Pierce, J. P., Natarajan, L., Caan, B. J., Parker, B. A., *et al*., Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: The Women's Healthy Eating and Living (WHEL) randomized trial. *JAMA* 2007, *298*, 289 – 298.

[20] Michels, K. B., Giovannucci, E., Chan, A. T., Singhania, R., *et al*., Fruit and vegetable consumption and colorectal adenomas in the Nurses' Health Study. *Cancer Res.* 2006, *66*, 3942 – 3953.

[21] Larsson, S. C., Bergkvist, L., Wolk, A., Fruit and vegetable consumption and incidence of gastric cancer: A prospective study. *Cancer Epidemiol. Biomarkers Prev.* 2006, *15*, 1998 – 2001.

[22] Casagrande, S. S., Wang, Y., Anderson, C., Gary, T. L., Have Americans increased their fruit and vegetable intake? The trends between 1988 and 2002. *Am. J. Prev. Med.* 2007, *32*, 257 – 263.

[23] Jeffery, E. H., Keck, A. S., *Isothiocyanates. Encyclopedias of Dietary Supplements*, Taylor and Francis, New York, NY 2006.

[24] Shen, G., Jeong, W. S., Hu, R., Kong, A. N., Regulation of Nrf2, NF-kappaB, and AP-1 signaling pathways by chemopreventive agents. *Antioxid. Redox Signal.* 2005, *7*, 1648 – 1663.

[25] Xiao, D., Lew, K. L., Zeng, Y., Xiao, H., *et al*., Phenethyl isothiocyanate-induced apoptosis in PC-3 human prostate cancer cells is mediated by reactive oxygen species-dependent disruption of the mitochondrial membrane potential. *Carcinogenesis* 2006, *27*, 2223 – 2234.

[26] Chiao, J. W., Chung, F. L., Kancherla, R., Ahmed, T., *et al*., Sulforaphane and its metabolite mediate growth arrest and apoptosis in human prostate cancer cells. *Int. J. Oncol.* 2002, *20*, 631 – 636.

[27] Wang, L. G., Liu, X. M., Chiao, J. W., Repression of androgen receptor in prostate cancer cells by phenethyl isothiocyanate. *Carcinogenesis* 2006, *27*, 2124 – 2132.

[28] Myzak, M. C., Hardin, K., Wang, R., Dashwood, R. H., *et al*., Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. *Carcinogenesis* 2006, *27*, 811 – 819.

[29] Myzak, M. C., Dashwood, R. H., Chemoprotection by sulforaphane: Keep one eye beyond Keap1. *Cancer Lett.* 2006, *233*, 208 – 218.

[30] Zhang, Y., Talalay, P., Cho, C. G., Posner, G. H., A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. *Proc. Natl. Acad Sci. USA* 1992, *89*, 2399 – 2403.

[31] Gasper, A. V., Al-Janobi, A., Smith, J. A., Bacon, J. R., *et al*., Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *Am. J. Clin. Nutr.* 2005, *82*, 1283 – 1291.

[32] Smith, T. K., Mithen, R., Johnson, I. T., Effects of Brassica vegetable juice on the induction of apoptosis and aberrant crypt foci in rat colonic mucosal crypts in vivo. *Carcinogenesis* 2003, *24*, 491 – 495.

[33] Khor, T. O., Hu, R., Shen, G., Jeong, W. S., *et al*., Pharmacogenomics of cancer chemopreventive isothiocyanate compound sulforaphane in the intestinal polyps of ApcMin/+ mice. *Biopharm. Drug Dispos*. 2006, *27*, 407 – 420.

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

[35] Al Janobi, A. A., Mithen, R. F., Gasper, A. V., Shaw, P. N., *et al*., Quantitative measurement of sulforaphane, iberin and their mercapturic acid pathway metabolites in human plasma and urine using liquid chromatography-tandem electrospray ionisation mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2006, *844*, 223 – 234.

[36] Fimognari, C., Nusse, M., Berti, F., Iori, R., *et al*., Isothiocyanates as novel cytotoxic and cytostatic agents: Molecular pathway on human transformed and non-transformed cells. *Biochem. Pharmacol.* 2004, *68*, 1133 – 1138.

[37] Fimognari, C., Nusse, M., Berti, F., Iori, R., *et al*., Sulforaphane modulates cell cycle and apoptosis in transformed and non-transformed human T lymphocytes. *Ann. NY Acad. Sci.* 2003, *1010*, 393 – 398.

[38] Musk, S. R., Johnson, I. T., Allyl isothiocyanate is selectively toxic to transformed cells of the human colorectal tumour line HT29. *Carcinogenesis* 1993, *14*, 2079 – 2083.

[39] Chan, K. C., Yin, M. C., Chao, W. J., Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. *Food Chem. Toxicol.* 2007, *45*, 502 – 507.

[40] Harris, K. E., Jeffery, E. H., Sulforaphane and erucin increase MRP1 and MRP2 in human carcinoma cell lines. *J. Nutr. Biochem.*, DOI: 10.1016/j.jnutbio.2007.02.014.

[41] Ovesen, L., Lyduch, S., Idorn, M. L., The effect of a diet rich in brussels sprouts on warfarin pharmacokinetics. *Eur. J. Clin. Pharmacol.* 1988, *34*, 521 – 523.

[42] Whitten, D. L., Myers, S. P., Hawrelak, J. A., Wohlmuth, H., The effect of St John's wort extracts on CYP3A: A systematic review of prospective clinical trials. *Br. J. Clin. Pharmacol.* 2006, *62*, 512 – 526.

[43] Higdon, J. V., Delage, B., Williams, D. E., Dashwood, R. H., Cruciferous vegetables and human cancer risk: Epidemiologic evidence and mechanistic basis. *Pharmacol. Res.* 2007, *55*, 224 – 236.

[44] Walters, D. G., Young, P. J., Agus, C., Knize, M. G., *et al*., Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in humans. *Carcinogenesis* 2004, *25*, 1659 – 1669.

[45] Pappa, G., Strathmann, J., Lowinger, M., Bartsch, H., *et al*., Quantitative combination effects between sulforaphane and 3,3′-diindolylmethane on proliferation of human colon cancer cells in vitro. *Carcinogenesis* 2007, *28*, 1471 – 1477.

[46] Martland, M. F., Butler, E. J., Fenwick, G. R., Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: The effects of feeding high and low glucosinolate meals. *Res. Vet. Sci.* 1984, *36*, 298 – 309.

[47] Cornblatt, B. S., Ye, L., Dinkova-Kostova, A. T., Erb, M., *et al*., Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis* 2007, *28*, 1485 – 1490.

[48] Wattenberg, L. W., Hanley, A. B., Barany, G., Sparnins, V. L., *et al*., Inhibition of carcinogenesis by some minor dietary constituents. *Princess Takamatsu Symp.* 1985, *16*, 193 – 203.

[49] Gills, J. J., Jeffery, E. H., Matusheski, N. V., Moon, R. C., *et al*., Sulforaphane prevents mouse skin tumorigenesis during the stage of promotion. *Cancer Lett.* 2006, *236*, 72 – 79.

[50] Gamet-Payrastre, L., Li, P., Lumeau, S., Cassar, G., *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.

[51] Kong, A. N., Yu, R., Hebbar, V., Chen, C., *et al*., Signal transduction events elicited by cancer prevention compounds. *Mutat. Res.* 2001, *480 – 481*, 231 – 241.

[52] Jackson, S. J., Singletary, K. W., Sulforaphane inhibits human MCF-7 mammary cancer cell mitotic progression and tubulin polymerization. *J. Nutr.* 2004, *134*, 2229 – 2236.

[53] Leoni, O., Iori, R., Palmieri, S., Esposito, E., *et al*., Myrosinase-generated isothiocyanate from glucosinolates: Isolation, characterization and in vitro antiproliferative studies. *Bioorg. Med. Chem.* 1997, *5*, 1799 – 806.

[54] Matusheski, N. V., Wallig, M. A., Juvik, J. A., Klein, B. P., *et al*., Preparative HPLC method for the purification of sulforaphane and sulforaphane nitrile from Brassica oleracea. *J. Agric. Food Chem.* 2001, *49*, 1867 – 1872.

[55] Matusheski, N. V., Jeffery, E. H., Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.* 2001, *49*, 5743 – 5749.

[56] Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D. J., *et al*., The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences Trichoplusia ni herbivory. *Plant Cell.* 2001, *13*, 2793 – 2807.

[57] Matusheski, N. V., Swarup, R., Juvik, J. A., Mithen, R., *et al*., Epithiospecifier protein from broccoli (Brassica oleracea L. ssp. italica) inhibits formation of the anticancer agent sulforaphane. *J. Agric. Food Chem.* 2006, *54*, 2069 – 2076.

[58] Cheng, D. L., Hashimoto, K., Uda, Y., In vitro digestion of sinigrin and glucotropaeolin by single strains of Bifidobacterium and identification of the digestive products. *Food Chem. Toxicol.* 2004, *42*, 351 – 357.

[59] Clapper, M. L., Szarka, C. E., Pfeiffer, G. R., Graham, T. A., *et al*., Preclinical and clinical evaluation of broccoli supplements as inducers of glutathione S-transferase activity. *Clin. Cancer Res.* 1997, *3*, 25 – 30.

[60] Zhao, B., Seow, A., Lee, E. J., Poh, W. T., *et al*., Dietary isothiocyanates, glutathione S-transferase-M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 1063 – 1067.

[61] Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K., *et al*., Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: Metabolism and excretion in humans. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 501 – 508.

[62] Kassahun, K., Davis, M., Hu, P., Martin, B., *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.

[63] Conaway, C. C., Getahun, S. M., Liebes, L. L., Pusateri, D. J., *et al*., Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr. Cancer* 2000, *38*, 168 – 178.

[64] Hu, R., Hebbar, V., Kim, B. R., Chen, C., *et al*., In vivo pharmacokinetics and regulation of gene expression profiles by isothiocyanate sulforaphane in the rat. *J. Pharmacol. Exp. Ther.* 2004, *310*, 263 – 271.

[65] Bradlow, H. L., Michnovicz, J., Telang, N. T., Osborne, M. P., Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* 1991, *12*, 1571 – 1574.

[66] Michnovicz, J. J., Adlercreutz, H., Bradlow, H. L., Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. *J. Natl. Cancer Inst.* 1997, *89*, 718 – 723.

[67] Fishman, J., Schneider, J., Hershcope, R. J., Bradlow, H. L., Increased estrogen-16alphahydroxylase activity in women with breast and endometrial cancer. *J. Steroid. Biochem.* 1984, *20*, 1077 – 1081.

[68] Kall, M. A., Vang, O., Clausen, J., Effects of dietary broccoli on human drug metabolising activity. *Cancer Lett.* 1997, *114*, 169 – 170.

[69] Hakooz, N., Hamdan, I., Effects of dietary broccoli on human in vivo caffeine metabolism: A pilot study on a group of Jordanian volunteers. *Curr. Drug Metab.* 2007, *8*, 9 – 15.

[70] Zhang, S. M., Hunter, D. J., Rosner, B. A., Giovannucci, E. L., *et al*., Intakes of fruits, vegetables, and related nutrients and the risk of non-Hodgkin's lymphoma among women. *Cancer Epidemiol. Biomarkers Prev.* 2000, *9*, 477 – 485.

[71] Kolonel, L. N., Hankin, J. H., Whittemore, A. S., Wu, A. H., *et al*., Vegetables, fruits, legumes and prostate cancer: A multiethnic case-control study. *Cancer Epidemiol. Biomarkers Prev.* 2000, *9*, 795 – 804.

[72] Fowke, J. H., Chung, F. L., Jin, F., Qi, D., *et al*., Urinary isothiocyanate levels, brassica, and human breast cancer. *Cancer Res.* 2003, *63*, 3980 – 3986.

[73] Yuan, J. M., Gago-Dominguez, M., Castelao, J. E., Hankin, J. H., *et al*., Cruciferous vegetables in relation to renal cell carcinoma. *Int. J. Cancer* 1998, *77*, 211 – 216.

[74] Nagle, C. M., Purdie, D. M., Webb, P. M., Green, A., *et al*., Dietary influences on survival after ovarian cancer. *Int. J. Cancer* 2003, *106*, 264 – 269.

[75] Bacon, J. R., Plumb, G. W., Howie, A. F., Beckett, G. J., *et al*., Dual action of sulforaphane in the regulation of thioredoxin reductase and thioredoxin in human HepG2 and Caco-2 cells. *J. Agric. Food Chem.* 2007, *55*, 1170 – 1176.

[76] Brooks, J. D., Paton, V. G., Vidanes, G., Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 949 – 954.

[77] Pledgie-Tracy, A., Sobolewski, M. D., Davidson, N. E., Sulforaphane induces cell type-specific apoptosis in human breast cancer cell lines. *Mol. Cancer Ther.* 2007, *6*, 1013 – 1021.

[78] Jakubikova, J., Sedlak, J., Mithen, R., Bao, Y., Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G2/M arrest and cell death in Caco-2 cells. *Biochem. Pharmacol.* 2005, *69*, 1543 – 1552.

[79] Herman-Antosiewicz, A., Xiao, H., Lew, K. L., Singh, S. V., Induction of p21 protein protects against sulforaphane-induced mitotic arrest in LNCaP human prostate cancer cell line. *Mol. Cancer Ther.* 2007, *6*, 1673 – 1681.

[80] Fimognari, C., Nusse, M., Cesari, R., Iori, R., *et al*., Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. *Carcinogenesis* 2002, *23*, 581 – 586.

[81] Choi, S., Lew, K. L., Xiao, H., Herman-Antosiewicz, A., *et al*., D,L-Sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. *Carcinogenesis* 2007, *28*, 151 – 162.

[82] Shan, Y., Sun, C., Zhao, X., Wu, K., *et al*., Effect of sulforaphane on cell growth, G(0)/G(1) phase cell progression and apoptosis in human bladder cancer T24 cells. *Int. J. Oncol.* 2006, *29*, 883 – 888.

[83] Chaudhuri, D., Orsulic, S., Ashok, B. T., Antiproliferative activity of sulforaphane in Akt-overexpressing ovarian cancer cells. *Mol. Cancer Ther.* 2007, *6*, 334 – 345.

[84] Tang, L., Li, G., Song, L., Zhang, Y., The principal urinary metabolites of dietary isothiocyanates, N-acetylcysteine conjugates, elicit the same anti-proliferative response as their parent compounds in human bladder cancer cells. *Anticancer Drugs* 2006, *17*, 297 – 305.

[85] Canene-Adams, K., Lindshield, B. L., Wang, S., Jeffery, E. H., *et al*., Combinations of tomato and broccoli enhance antitumor activity in dunning r3327-h prostate adenocarcinomas. *Cancer Res.* 2007, *67*, 836 – 843.

[86] Hu, R., Khor, T. O., Shen, G., Jeong, W. S., *et al*., Cancer chemoprevention of intestinal polyposis in ApcMin/+ mice by sulforaphane, a natural product derived from cruciferous vegetable. *Carcinogenesis* 2006, *27*, 2038 – 2046.

[87] Dinkova-Kostova, A. T., Jenkins, S. N., Fahey, J. W., Ye, L., *et al*., Protection against UV-light-induced skin carcinogenesis in SKH-1 high-risk mice by sulforaphane-containing broccoli sprout extracts. *Cancer Lett.* 2006, *240*, 243 – 252.

[88] Fahey, J. W., Zhang, Y., Talalay, P., Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 10367 – 10372.

[89] Shapiro, T. A., Fahey, J. W., Dinkova-Kostova, A. T., Holtzclaw, W. D., *et al*., Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: A clinical phase I study. *Nutr. Cancer* 2006, *55*, 53 – 62.

[90] Ye, L., Dinkova-Kostova, A. T., Wade, K. L., Zhang, 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.