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Phytochemicals from Cruciferous Plants Protect against Cancer by Modulating Carcinogen Metabolism[1,2,3]

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ABSTRACT Several epidemiologic studies suggest that consumption of cruciferous vegetables may be particularly effective (compared with total fruit and vegetable consumption) in reducing cancer risk at several organ sites. Crucifers that are widely consumed are especially rich in glucosinolates, which are converted by plant myrosinase and gastrointestinal microflora to isothiocyanates. A number of isothiocyanates and a limited number of glucosinolates that were examined effectively block chemical carcinogenesis in animal models. Many isothiocyanates are also potent inducers of phase 2 proteins. Substantial evidence supports the view that phase 2 enzyme induction is a highly effective strategy for reducing susceptibility to carcinogens. This conclusion has recently received strong molecular support from experiments on mice in which the specific transcription factor, nrf2, which is essential for induction of phase 2 proteins, was deleted. In these knock-out mice, the basal levels of phase 2 enzymes are very low and not inducible. Accordingly, these mice are much more susceptible than their wild-type counterparts to benzo[a]pyrene forestomach carcinogenesis and are not protected by phase 2 inducers. These experiments provide very strong evidence for a major role of phase 2 enzymes in controlling the risk of exposure to carcinogens. An increasing number of phase 2 proteins that exert a variety of protective mechanisms are being identified. Thus, in addition to detoxifying electrophiles, these proteins exercise versatile, long-lasting and catalytic antioxidant protection. J. Nutr. 131: 3027S–3033S, 2001.

KEY WORDS: • *chemoprotection* • *sulforaphane* • *phase 2 enzyme induction* • *brassica vegetables* • *glucosinolates* • *isothiocyanates* • *antioxidants*

Earlier diagnosis, improvements in treatment and reduction in smoking are probably responsible for the steady although modest decreases in the age-adjusted cancer mortality in the United States in the past 10 y. Nevertheless, it is widely agreed that the number of new cases of cancer is rising worldwide, and the prognosis for patients afflicted with the major solid tumors (breast, lung, colorectal, prostate) in the Western world unfortunately remains dismal (1). The aging of populations and the dramatic successes in treatment of cardiovascular disease are shifting our terminal disease burden toward malignancy. If present trends continue, the estimated annual incidence of new cancer cases will double in 30 y (2). It is therefore inescapable that the long-term management of cancer requires concerted efforts to reduce the risk of cancer while continuing the intensive search for more effective treatments.

Risk reduction encompasses the following two strategies: *1*) prevention, i.e., the reduction in exposure to carcinogens (such as smoking or radiation); and *2*) protection, i.e., the deliberate intervention to enhance mostly endogenous mechanisms that reduce the risk arising from exposure to carcinogens. The principal carcinogenic agents are exogenous or metabolically generated electrophiles and reactive oxygen species arising from normal oxidative processes and from the environment. Although the necessity of eliminating, or at least reducing exposure to carcinogens is axiomatic, the challenges of protecting DNA from damage by carcinogens in healthy or even high-risk populations is formidable. Long-term or even life-long protection is required and this could be most easily and safely accomplished by identifying and administering chemoprotective agents of low toxicity that are already present in the human diet.

In this review, we summarize the evidence that cruciferous

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vegetables play a major, perhaps unique role in the widely recognized protective effects of vegetables against the risk of cancer. We develop support for the view that regular consumption of cruciferous vegetables leads to high intake of unusual phytochemicals known as glucosinolates and consequently exposure of cells to isothiocyanates, the products of glucosinolate hydrolysis. Isothiocyanates are well-known protectors against carcinogenesis and modulators of the activities of enzymes involved in the metabolism of carcinogens, especially by the induction of phase 2 detoxication enzymes. We present recent molecular evidence for the central role of phase 2 enzymes in determining susceptibility to carcinogens and that their induction reduces this susceptibility.

Protection against cancer risk by plant-rich diets

Consensus has been building over more than a quarter of a century that diets rich in fruits and vegetables are associated with lower risks of developing various types of malignancies. This consensus is supported by a growing number of sophisticated epidemiologic studies. Major reviews of this field include two reports from the National Academy of Sciences of the United States, i.e., *Diet, Nutrition, and Cancer* in 1982 (3) and *Diet and Health. Implications for Reducing Chronic Disease Risk* in 1989 (4), and the recent encyclopedic compendium produced by the World Cancer Research Fund and the American Institute for Cancer Research in 1997, *Food, Nutrition, and the Prevention of Cancer: A Global Perspective* (5). The classic paper by Doll and Peto (6) provided quantitative estimates of the avoidable risks of cancer in the United States. Although Doll and Peto focused largely on the causes of human cancer, they drew attention to the highly preventable nature of the disease and therefore issued a clarion call to action. Other comprehensive reviews of the relation of diet to cancer have been provided by Ziegler (7), Block and colleagues (8), and by Steinmetz and Potter (9–11).

Of >200 case-control and cohort studies, nearly 80% have reported significant inverse relations between consumption of plant foods and the risk of developing most types of cancer (5). Although conclusions with respect to the overall extent to which diet contributes to cancer incidence, or to be more explicit, the degree to which dietary modification might be expected to reduce cancer risk, vary considerably, a reasonable estimate is 30–40% (5).

Central role of cruciferous plants in protection against cancer

Multiple mechanisms are undoubtedly involved in the protective effects of diets rich in fruits and vegetables (5,9–11). These depend not only on qualitative and quantitative changes in major nutrient and nonnutrient dietary components, such as the reduction in meat and fat intake and corresponding increase in fiber consumption, but also changes in the intake of essential nutrients. Far less well understood are the effects of chronic consumption of substantial quantities of nonnutrient plant components, including a myriad of unique phytochemicals that plants accumulate, sometimes to substantial levels, for their own needs (12). It is therefore very difficult to identify the relative contributions of various components of a plant-based diet to overall cancer risk reduction. The issue is further complicated by the recent demonstration of synergism among protectors (13,14). This phenomenon is not unexpected and is analogous to the well-recognized and clinically important synergisms among chemotherapeutic agents, and the observations of more than additive effects among multiple carcinogens (e.g., asbestos, smoking and alcohol for lung cancer; aflatoxin and viral hepatitis for liver cancer).

In attempting to identify the relative importance of various mechanisms, one potentially important clue may be the growing evidence that among vegetables, cruciferous plants are especially effective as protectors (15). The Cruciferae (also known as the Brassicaceae) are the family of plants that include the various familiar members of the species *Brassica oleracea* (e.g., broccoli, cabbage, cauliflower, kale, Brussels sprouts) as well as many other plants that are widely consumed in various parts of the world but not in the United States, such as oriental cabbage, arugula, watercress, radish, daikon, wasabi and various mustards. Regional patterns of crucifer consumption vary substantially in different parts of the world; a striking example is the huge consumption of daikon (*Raphanus sativus*; 20 kg/y or 55 g/d) in Japan, where it is the most popular vegetable.

Epidemiologic evidence for the relationship between crucifer consumption and cancer risk. Epidemiologic evidence relating cancer risk reduction to the consumption of specific types of fruits and vegetables and to crucifers in particular has been available for >20 y. In 1978, Graham and colleagues (16) concluded: "a dose-response relationship was also encountered in analyses of each of the following for cancer of the colon: sauerkraut, coleslaw, Brussels sprouts, broccoli." Recent comprehensive reviews by Dutch workers (15,17) of numerous studies purporting to show a specific protective effect of crucifers, and especially brassicas, have cautioned: "It is not yet possible to decide whether the protective effect is attributable to brassica vegetables per se or to vegetables in general" (17). Since these reviews were published, further studies continue to report an inverse association between crucifer consumption and cancer. Jain et al. (18) and Kolonel et al. (19) observed highly significant cancer risk reduction with increasing crucifer intake in cohorts that developed prostate cancer. Recently, Terry et al. (20) reported reduction in breast cancer risk related to crucifer consumption, and Zhang et al. (21) observed crucifer-associated reduction in non-Hodgkin's lymphoma in women.

Two other recent studies attempted to analyze the specific protective role of crucifers. Michaud and colleagues (22) analyzed 252 cases of bladder cancer that developed in 47,909 health professionals during a 10-y period. No significant associations were found between bladder cancer risk and the consumption of total fruits and vegetables, fruits only, vegetables only, yellow vegetables or green leafy vegetables. However, the multivariate risk reduction (RR) ratio for cruciferous vegetables was highly significant (RR = 0.49, $P = 0.008$) (**Table 1**). Similarly, Cohen et al. (23) examined the relation between fruit and vegetable consumption and prostate cancer incidence in men <65 y of age. High fruit consumption did not affect prostate cancer incidence. Although high overall vegetable consumption was associated with reduced risk, cruciferous vegetables were clearly protective when risk was adjusted for total vegetable consumption and other variables.

These results highlight the great importance of obtaining reliable quantitative information on the consumption of individual or groups of plants and their phytochemical composition, preferably by direct chemical measurements, and relating these to cancer risk as well as determining the genotypes and phenotypes of the target populations (24,25). An example of the successful linking of a chemical index of crucifer consumption to the risk of lung cancer is described below (26). Reliable methods now available for quantifying crucifer consumption by analysis of urine must be applied more widely in epidemiologic studies (27–29).

TABLE 1

Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort[1]

	Relative risk	*P*-value
Fruits and vegetables	0.75	0.25
Fruits	1.12	0.68
Vegetables	0.72	0.09
Yellow vegetables	1.01	0.50
Green leafy vegetables	0.99	0.81
Cruciferous vegetables	0.49	0.008[2]

[1] Data from Michaud et al. (22); 252 cases of bladder cancer in 47,909 men in Health Professionals Follow-up Study (1986–1996).

[2] The only significant reduction in relative risk was observed with cruciferous vegetables.

The unusual phytochemistry of crucifers: glucosinolates and isothiocyanates. A striking and characteristic chemical property of cruciferous plants is their high content of glucosinolates, which often approaches 1% or more of their dry weight (30). Glucosinolates and their isothiocyanate hydrolysis products are well-known protectors against carcinogenesis, as will be discussed below. The relatively large consumption of glucosinolates by many individuals, in comparison with other plants currently under study as potential sources of chemoprotective activity, adds special significance to these compounds. Glucosinolates are β-thioglucoside *N*-hydroxysulfates (**Fig. 1**) and are the precursors of isothiocyanates (mustard oils) (31). Glucosinolates play protective and evolutionarily important roles in plants. These include allelopathy (suppression of growth of neighboring plants), specific positive and negative feeding cues for some insects and broad antibiotic properties including nematocidal, antimicrobial, antifungal, antiprotozoal and insecticidal activities. Glucosinolates are invariably accompanied in plant cells by the enzyme myrosinase (a β-thioglucosidase), which is normally physically segregated from its glucosinolate substrates but is released and hydrolyzes glucosinolates to isothiocyanates and other products when plants are injured by predators or when food is prepared or chewed (Fig. 1). This reaction is responsible for the development of the sharp taste of horseradish, mustard and wasabi. In the absence of myrosinase, for example, when plants are cooked and myrosinase is heat inactivated, humans can efficiently convert glucosinolates to isothiocyanates through the action of the microflora of the gastrointestinal tract (28,29).

At least 120 chemically distinct glucosinolates have been identified in plants (31). Although the majority have been isolated from crucifers, 15 other families of plants are known to contain glucosinolates. The other families include many edible species, and although they are unlikely to contribute significantly to human glucosinolate intake in the Western world, their significance for chemoprotection in other parts of the world is yet to be determined. Although only few attempts have been made to assess human glucosinolate consumption, some estimates are as high as 300 mg/d (~660 μmol/d) (32).

Chemoprotective effects of isothiocyanates and glucosinolates. Since the early 1960s, both natural and synthetic isothiocyanates have attracted considerable and growing attention as important and effective protectors against chemical carcinogenesis in a number of animal models (33–36). Although only a few glucosinolates have been examined, largely because adequate quantities of these compounds have been unavailable, some are very effective in inhibiting carcinogenesis (37–39). Interest in the use of isothiocyanates as chemoprotectors arose from several largely independent but now converging directions. The history of these developments can be traced from the comprehensive review by Hecht (35), who also summarizes the potential mechanisms underlying the protective effects of these compounds. Many different (≥25) isothiocyanates block the carcinogenic effects of more than a dozen chemically different types of carcinogens in a least 10 different target sites in three species of rodents. The earliest experiments dating back to the 1960s involved the use of α- and β-naphthyl isothiocyanates as inhibitors of carcinogenesis. The most extensive work has been done by Hecht and his colleagues on the tobacco-specific nitrosamine carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is probably the most prominent carcinogen derived from cigarettes (35). Several isothiocyanates inhibit the action of this carcinogen through inhibition of its metabolism.

In light of the substantial consumption of crucifers by many humans, as mentioned above, it is tempting to attribute the growing evidence that crucifers play a special role in protection against cancer to their unique chemistry, most notably their very high levels of glucosinolates, which are efficiently converted to isothiocyanates. With the recent development in our laboratory of a simple spectroscopic method (28,29,40,41) for the quantitative determination of isothiocyanates, glucosinolates (after myrosinase hydrolysis) and their major urinary metabolites (dithiocarbamates) by cyclocondensation with 1,2-benzene-dithiol, it has become feasible to assess crucifer consumption, use it as an epidemiologic tool and relate it to cancer risk.

London and colleagues (26) found a significant association between the presence of dithiocarbamates (which are glucosinolate and isothiocyanate metabolites) in the urine of a large cohort of men in Shanghai and their subsequent risk of developing lung cancer. Contrary to the title of this paper, the analytes measured in the urine are dithiocarbamate metabolites because the levels of isothiocyanates in urine are negligible (28,29,42). Those who excreted dithiocarbamates, an index of glucosinolate and isothiocyanate consumption, had a lower risk. This protective effect became more prominent in individuals with homozygous deletions in certain glutathione transferases (M1 and T1). Because these enzymes are involved in the conversion of isothiocyanates to dithiocarbamates and presumably facilitate the excretion of isothiocyanates, the findings suggest that the activities of these enzymes lower effective tissue levels of isothiocyanates. These findings provide additional support for the pivotal role of the glucosinolates and isothiocyanates derived from crucifers in chemoprotection against cancer.

Role of phase 2 enzymes in chemoprotection

Carcinogen metabolism by phase 1 and phase 2 enzymes. Two types of DNA-damaging agents can evoke neoplastic transformations, i.e., electrophiles, largely of exogenous origin,

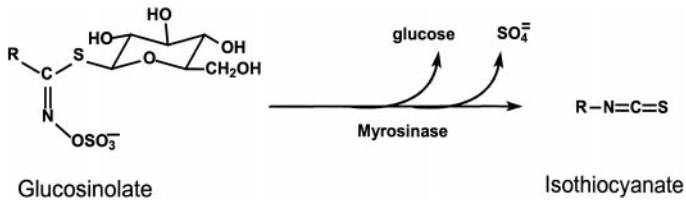

FIGURE 1 Conversion of glucosinolates to isothiocyanates by plant myrosinase.

and reactive oxygen species, originating in part from exogenous sources but arising also in substantial quantities from normal cellular oxidations. As shown in **Figure 2**, most electrophiles require metabolic activation, usually by phase 1 enzymes (cytochromes P_{450}); they convert generally innocuous procarcinogens to highly reactive electrophilic ultimate carcinogens that can damage susceptible centers of DNA bases and initiate carcinogenesis. DNA and other macromolecules are principally protected against damage by electrophiles and reactive oxygen species by a family of phase 2 enzymes. By a variety of mechanisms (discussed below) including conjugation with endogenous ligands (e.g., glutathione, glucuronic acid), phase 2 enzymes inactivate these agents and promote their excretion. In addition, glutathione, the principal and most abundant small-molecule cellular antioxidant, which is similarly regulated by phase 2 enzymes, plays a major role in protection against electrophiles and reactive oxygen species. Thus, whether malignancy will ensue when a cell is exposed to a potential carcinogen is determined largely by the balance of activities of phase 1 enzymes that activate carcinogens and phase 2 enzymes that nearly always detoxify reactive carcinogens. It is therefore of considerable importance that both families of enzymes are highly inducible in many tissues and that their activities can be regulated by a wide variety of chemical agents belonging to nine chemical classes (43–46), among which dietary phytochemicals are especially important. Furthermore, although some inducers elevate both phase 1 and phase 2 enzymes (bifunctional inducers), others selectively induce only phase 2 enzymes (monofunctional inducers) (47).

Evidence that induction of phase 2 enzymes results in chemoprotection. Many lines of evidence support the importance of phase 2 enzymes in regulating susceptibility to carcinogens (48–51). Our early findings that administration of phenolic antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) resulted in the induction of phase 2 enzymes by enhanced transcription in many rodent tissues led to the suggestion that enhanced activities of these detoxication enzymes were responsible for these protective actions. Evidence for this assertion has been growing during the past 20 y and has been summarized elsewhere (48–51). Among the most persuasive considerations is that compounds isolated from natural sources solely on the basis of their inducer activity have subsequently been shown to protect rodents against carcinogenesis (e.g., sulforaphane, terpenes from green coffee beans, resveratrol) and that other compounds were predicted to have chemoprotective activity based on their phase 2 enzyme inducer properties (e.g., oltipraz and other 1,2-dithiole-3-thiones, and a series of synthetic analogs of sulforaphane). Indeed, a voluminous literature now supports the view that induction of phase 2 enzymes is an important and sufficient mechanism for achieving protection against the toxic and neoplastic effects of many carcinogens (48–50,52). These considerations have guided the use of oltipraz (a phase 2 enzyme inducer) in reducing the risk of aflatoxin exposure in a region of China in which the population is afflicted with a very high incidence of liver cancer. Oltipraz promoted aflatoxin B_1 excretion largely through induction of phase 2 enzymes (53).

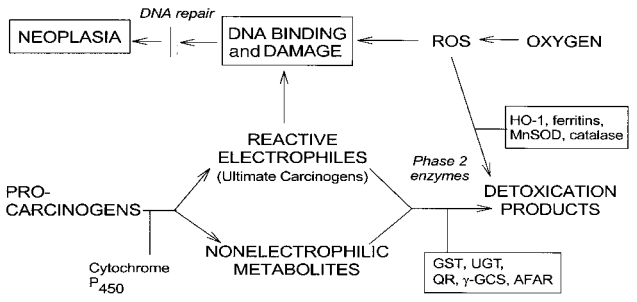

FIGURE 2 Role of metabolism in chemical carcinogenesis. Susceptibility to carcinogen damage is controlled by the balance between phase 1 activation and phase 2 detoxication enzymes. GST, glutathione *S*-transferases; γ-GCS, γ-glutamylcysteine synthase; HO-1, heme oxygenase 1; MnSOD, manganese superoxide dismutase; QR, quinone reductase (NQO1); ROS, reactive oxygen species; UGT, UDP-glucuronosyl transferases; AFAR, aflatoxin B_1 aldehyde reductase.

Distribution of phase 2 enzyme inducers among plants: isolation of sulforaphane. Recognizing the potential importance of phase 2 enzyme inducer potency determinations as a strategy for identifying anticarcinogens, Prochaska and Santamaria (54) devised a simple system for measuring quinone reductase specific activities in murine hepatoma cells grown in 96-well microtiter plates. Quinone reductase was selected as a prototype for phase 2 enzymes because of its widespread distribution in mammalian systems, large amplitude of inducer response and ease of measurement by coupling to tetrazolium dye reduction. This system provides a highly quantitative and reproducible method for determining inducer potencies of pure compounds, mixtures or plant extracts (38,55) and even urinary metabolites (29). The results obtained with this system have reliably predicted the behavior of inducers in animals.

When organic extracts of various edible plants belonging to several plant families were examined for phase 2 inducer activities, striking differences were observed (55). Thus Cruciferae, and particularly the brassicas, were especially rich in inducer activities, whereas many other plant families were generally much poorer sources. We tested a large number of items from a special balanced diet designed for clinical studies in which it was important to minimize the intake of inducers. Organic solvent extracts of the many components of this diet, including meats, fruits, noncruciferous vegetables, grains and a variety of dairy products, contained much less than 1% of the inducer activity per gram than did the equivalent weight of an average head of broccoli (35,000 U/g) when measured by our standard assay.

The importance of developing glucosinolates and isothiocyanates as chemoprotectors received considerable impetus from the totally independent and unexpected bioassay-guided discovery that the principal inducer of phase 2 detoxication enzymes in broccoli, and especially in 3-d-old broccoli sprouts, was an unusual isothiocyanate, i.e., sulforaphane [1-isothiocyanato-(4*R*)-methylsulfinyl)-butane; $CH_3S(CH_2)_4NCS$] that blocked mammary tumor formation in rats treated with dimethylbenz[a]anthracene (DMBA) (38,40,56). Sulforaphane is an extremely potent inducer of phase 2 enzymes, perhaps the most potent naturally occurring inducer described to date. Further support for the anticarcinogenic activity of isothiocyanates was afforded by the synthesis of a large number of isothiocyanate analogs on the basis of their potencies as phase 2 enzyme inducers and the finding that these compounds also inhibited mammary tumor formation in rats evoked by DMBA (57).

Molecular evidence for critical importance of phase 2 enzymes in regulating carcinogenesis: evidence obtained from disruption of the* nrf2 *gene. Additional and more complete evidence for the importance of phase 2 enzymes in regulating susceptibility to carcinogens and mediating chemoprotection has now been obtained by specific gene deletion. Many mono-

TABLE 2

Effect of phase 2 enzyme induction by oltipraz on neoplasia of forestomach in female wild-type and nrf2-deficient mice[1]

Genotype of mouse	Animals *n*	Protective treatment	Gastric tumors/mouse	*P*-value
Wild type	14	None	9.5 ± 1.0	0.003
Wild type	18	Oltipraz	4.6 ± 0.5	0.011
nrf2 deficient	14	None	14.1 ± 1.2	0.983
nrf2 deficient	16	Oltipraz	13.6 ± 1.1	

[1] Data from Ramos-Gomez et al. (64). Female wild-type and *nrf2*-deficient mice received four treatments at 1-wk intervals with benzo[a]pyrene. Oltipraz was administered 48 h before each treatment. Number of tumors (±SEM) are reported per mouse at termination of experiment (30 wk after first carcinogen treatment).

functional inducers (54), which selectively elevate phase 2 enzymes without inducing phase 1 enzymes, appear to do so by activating antioxidant response elements (ARE) located in the 5′-upstream region of many of these enzymes (44,45,52,58,59). Yamamoto and colleagues (60,61) recently described an important mechanism of regulation of the ARE element by inducers that involves participation of Nrf2, a member of the basic leucine zipper family of transcription factors. The binding of Nrf2 to ARE signals the transcription of genes coding for phase 2 enzymes. Under basal conditions, Nrf2 is anchored in the cellular cytosol primarily by binding to the chaperone Keap1, which is itself tethered to actin fibers. In the presence of phase 2 inducers, this combination is disrupted and Nrf2 migrates to the nucleus, where in dimeric combination with other transcription factors such as small Mafs, it binds to the ARE and activates phase 2 gene transcription, resulting in increased synthesis of the cognate enzymes.

Recent experiments (60–63) showed that mice in which the *nrf2* gene was deleted had lower levels of glutathione transferases, quinone reductase and other phase 2 enzymes as well as depressed glutathione-synthesizing enzymes in a number of tissues; as expected, these enzymes were essentially not inducible by a variety of phase 2 inducers. When *nrf2* gene knock-out mice received benzo[a]pyrene by gavage, they developed 50% more tumors than did their wild-type controls. Administration of oltipraz (an inducer) reduced the tumor multiplicity in the wild-type mice by >50%, whereas this compound was completely ineffective in the mutant *nrf2* gene knock-out mice. (64). These differences were significant (**Table 2**). These experiments not only provide long-awaited proof, based on molecular genetics, that phase 2 enzymes play a critical role in determining susceptibility to carcinogens and that their induction leads to decreased susceptibility, but also suggest that phase 2 enzyme induction is a major defense strategy, at least in the model examined. Notably, the tumor incidence in the protected wild-type mice (4.6 tumors/mouse) is less than one third that of the *nrf2* gene–deficient mice (14.1 tumors/mouse).

The phase 2 response: mechanisms by which phase 2 enzymes protect against cancer. In 1967, Williams (65) formally suggested that the metabolism of xenobiotics be considered as involving the tandem actions of two families of enzymes, i.e., phase 1 and phase 2. Phase 1 enzymes made hydrophobic compounds functional largely by oxidations and reductions, whereas phase 2 enzymes promoted the conjugation of the phase 1 products with endogenous ligands such as glutathione (by glutathione *S*-transferases) and glucuronic acid (by UDP-glucuronosyltransferases), resulting in more water-soluble products that could be easily excreted. Extensive studies of these enzymes have shown that the conjugating enzymes are induced by a wide variety of synthetic and natural chemical agents coordinately with a large number of other

TABLE 3

Protective functions of inducible phase 2 proteins

Inducible phase 2 proteins	Protective mechanisms	Reference
Glutathione *S*-transferases (α, μ, π)	Conjugate with glutathione (GSH) Reduce alkyl, lipid, and DNA base hydroperoxides	52, 66–69
UDP-glucuronosyltransferases	Conjugate with glucuronic acid	70
NAD(P)H:quinone oxidoreductase (QR, NQO1)	Reduce quinones to hydroquinones. Prevent oxidative cycling. Regenerate Coenzyme Q, Vitamin E	71–73
Epoxide hydrolases	Hydrolyze epoxides	70
Dihydrodiol dehydrogenase	Converts dihydrodiols to catechols	70
γ-Glutamylcysteine synthetase	Increases GSH levels	52, 74, 75
Glutathione conjugate efflux pumps	Expels GSH conjugates from cells	52, 76
Heme oxygenase 1	Generates antioxidants (bilirubin, CO)	77–79
Ferritin (heavy and light subunits)	Sequesters free ferrous iron	78
Manganese superoxide dismutase	Reduces superoxide levels	
Catalase	Reduces hydrogen peroxide levels	
Aflatoxin B_1 aldehyde reductase	Reduces reactive metabolite	52
Leukotriene B_4 dehydrogenase	Depletes leukotriene B_4 and reduces inflammatory reaction	80

enzymes (48–51). Although the induction patterns vary quantitatively in many tissues, they also show many similarities. Thus the restricted view of phase 2 enzymes as promoting conjugation reactions has evolved into a much broader appreciation of their functional scope and importance. We suggest that these inductions be designated the *phase 2 response* and be defined by the following features: *1*) coordinate induction by several representatives of the same chemical classes of compounds that also induce classical phase 2 enzymes (glutathione *S*-transferases and UDP-glucuronosyltransferases); *2*) regulation by mechanisms that are similar and may involve common promoters and transcription factors (e.g., ARE and Nrf2, respectively); and *3*) catalysis of a broad range of other chemical reactions that protect cells against the toxic and neoplastic effects of electrophiles and reactive oxygen species (50). **Table 3** provides a partial list of the enzymes and other proteins that currently conform to these definitions of the phase 2 response.

With the progressive identification of more participants in the phase 2 response, the view is emerging that the induced proteins play important and broad roles not only in the detoxication of electrophiles (45) but also as a highly important component of the antioxidant defenses of cells (81). In combating oxidative stress, the phase 2 response differs in important ways from the antioxidant activities of small molecules such as vitamin C and the tocopherols. The phase 2 response is catalytic, has prolonged action (governed by the half-life of the proteins), is chemically versatile and is unlikely to produce prooxidant effects. The phase 2 response is therefore emerging as a very important component of cellular defenses against oxidants.

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