Eur J Nutr (2008) 47 (Suppl 2):73–88
DOI 10.1007/s00394-008-2009-8

John D. Hayes
Michael O. Kelleher
Ian M. Eggleston

The cancer chemopreventive actions of phytochemicals derived from glucosinolates

Abstract This article reviews the mechanisms by which glucosinolate breakdown products are thought to inhibit carcinogenesis. It describes how isothiocyanates, thiocyanates, nitriles, cyanoepithioalkanes and indoles are produced from glucosinolates through the actions of myrosinase, epithiospecifier protein and epithiospecifier modifier protein released from cruciferous vegetables during injury to the plant. The various biological activities displayed by these phytochemicals are described. In particular, their abilities to induce cytoprotective genes, mediated by the Nrf2 (NF-E2 related factor 2) and AhR (arylhydrocarbon receptor) transcription factors, and their abilities to repress NF-κB (nuclear factor-κB) activity, inhibit histone deacetylase, and inhibit cytochrome P450 are outlined. Isothiocyanates appear to alter gene expression through modification of critical thiols in regulatory proteins such as Keap1 (Kelch-like ECH-associated protein 1) or IKK (IκB kinase), causing activation of Nrf2 and inactivation of NF-κB, respectively. Certain indoles act as ligands for AhR. Isothiocyanates and indoles are also capable of affecting cell cycle arrest and stimulating apoptosis. The mechanisms responsible for these anti-proliferative responses are discussed.

Key words antioxidant response element – apoptosis – arylhydrocarbon receptor – cytochrome P450 – epithionitriles – gene induction – glucosinolates – glutathione *S*-transferase – isothiocyanates – NF-κB – Nrf2 – quinone reductase – xenobiotic response element

J.D. Hayes (✉) · M.O. Kelleher
Biomedical Research Centre
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY
Scotland, UK
E-Mail: j.d.hayes@dundee.ac.uk

I.M. Eggleston
Dept. of Pharmacy
and Pharmacology
University of Bath
Bath, UK

Glucosinolates and their association with cancer chemoprevention

Regular consumption of cruciferous vegetables, such as broccoli, Brussels sprouts, cabbage, cauliflower, kale, swede and turnip, is associated with a reduced incidence of cancer [129, International Agency for Research on Cancer Workgroup [55]]. Furthermore, greater health benefit may be obtained from raw as opposed to cooked vegetables [72]. In man, these vegetables appear to protect against colorectal cancer [104], lung cancer [73], and possibly prostate cancer [42]. In animals, feeding experiments have also suggested broccoli can protect against liver cancer [109]. Cruciferous vegetables uniquely contain glucosinolates at approximately 20 μmol/g dry mass of vegetable [23, 66], and it is thought that these phytochemicals are primarily responsible for the putative cancer chemoprevention conferred by eating diets that contain significant quantities of these vegetables [37, 59].

Glucosinolates are substituted β-thioglucoside *N*-hydroxysulfates, formed by the plant from any one of eight amino acids, namely, alanine, valine, leucine, isoleucine, phenylalanine, methionine, tyrosine and

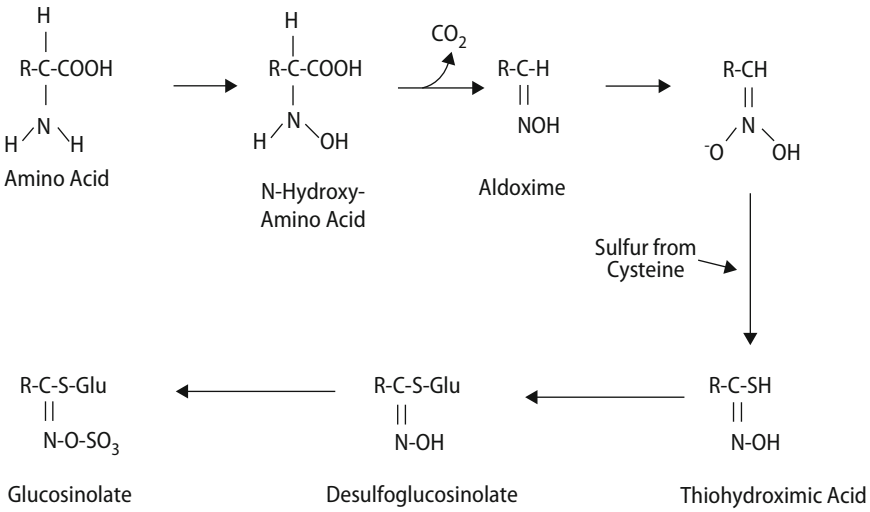

Fig. 1 Synthesis of Glucosinolates. The R group is derived from the original amino acid (i.e. Ala, Val, Leu, Ile, Phe, Met, Tyr or Trp) and is highly variable

tryptophan [55]. Over 115 naturally occurring glucosinolates have been identified. Each cruciferous vegetable contains a mixture of glucosinolates that varies according to the strain of the plant [23, 35, 74, 89, 110]. The glucosinolate content is primarily under genetic control, with the last step in the pathway being of particular importance [128], though it can also be influenced by environmental factors [15, 36]. Much of the diversity amongst glucosinolates arises from the addition of different sized alkyl groups to the side chain of amino acids, principally valine, phenylalanine and methionine, used in their biosynthesis; this variable elongation of amino acid side chains entails repetitive additions of methyl groups through a series of transamination, condensation, isomerisation and decarboxylation reactions [43]. As shown in Fig. 1, the synthesis of glucosinolates proceeds through the conversion of elongated amino acids to their oxime derivatives, catalysed by members of the cytochrome P450 (CYP) 79 family [3]. Subsequently, the oxime is metabolised to a thiohydroximate, which is in turn conjugated with glucuronic acid to form a desulfoglucosinolate before finally being sulfated to yield the glucosinolate [55].

The task of establishing a link between the ingestion of particular glucosinolates and their possible health benefits is not straightforward. This endeavour is simplified to some extent by the fact that relatively few glucosinolates are present in the human diet. The most common of these are the methylsulfinylalkyl glucosinolates glucoiberin and glucoraphanin, the olefinic glucosinolates sinigrin, gluconapin, glucobrassicanapin and progoitrin, and the aromatic glucosinolate gluconasturtiin (Table 1) [66, 118]. Glucoraphanin has been reported to be abundant in broccoli [66], though certain strains of this plant also contain substantial amounts of glucoiberin [87]. Sinigrin has been reported to be the predominant glucosinolate in Brussels sprouts, cabbage, cauliflower and kale [66]; gluconapin is also found in high levels in Brussels sprouts [66]. Substantial amounts of progoitrin are present in many cruciferous vegetables [66]. The aromatic glucosinolate gluconasturtin is present in watercress. The indolyl glucosinolate glucobrassicin is present in Savoy cabbage, Brussels sprouts and cauliflower [66, 83], and whilst not abundant it can elicit distinct pharmacological effects.

Table 1 Trivial names of some glucosinolates with the corresponding side-chain (R) composition

Name	R side-chain
Sinigrin	2-Propenyl
Gluconapin	3-Butenyl
Glucobrassicin	3-Indolylmethyl
Glucobrassicanapin	4-pentenyl
Progoitrin	2-Hydroxy-3-butenyl
Glucoiberin	3-Methylsulfinylpropyl
Gluconapoleiferin	2-Hydroxy-4-pentenyl
Glucocheirolin	3-Methylsulfonylpropyl
Glucoerucin	4-Methylthiobutyl
Glucoberteroin	5-Methylthiopentyl

Production of isothiocyanates, thiocyanates, nitriles, cyano-epithioalkanes and oxazolidine-2-thiones from glucosinolates

Inhibition of carcinogenesis by glucosinolates is not primarily attributable to this class of compound, but rather it appears to be due to certain of their break-

down products. Hydrolysis of these phytochemicals is catalysed by myrosinase (*β*-thioglucoside glucohydrolase, EC 3.2.3.1), an enzyme that is physically segregated from glucosinolates within the intact plant by virtue of the fact that it is sequestered in specialised "myrosin" cells [5]. Upon wounding of the vegetable, for example during harvesting, during freeze–thawing, during food preparation, or during chewing whilst being eaten, myrosinase is released from the "myrosin" cells and catalyses the hydrolysis of glucosinolates within the damaged plant. In addition, myrosinase activity may be present in human colonic microflora, suggesting that it is possible glucosinolates are hydrolysed in the gastrointestinal tract during digestion of food [6, 32, 65]. Myrosinase cleaves glucosinolates at the thioglycoside linkage to produce glucose and an unstable aglycone thiohydroximate-*O*-sulfonate that spontaneously rearranges to yield several breakdown products. The outcome of the reaction with myrosinase depends on the nature of the aglycone, as well as the reaction temperature, the pH and the presence of ferrous ions (Fig. 2).

The thiohydroximate-*O*-sulfates formed from methylsulfinylalkyl, olefinic and aromatic glucosinolates undergo a Lossen rearrangement, with the elimination of sulfate, to form their respective isothiocyanates (ITCs), thiocyanates or nitriles [6, 37]. Elemental sulfur is also formed in certain circumstances. At neutral pH, hydrolysis of glucosinolates with aliphatic or aromatic side chains gives rise primarily to ITCs. The glucosinolates glucoiberin, gluconapin, glucoraphanin, glucobrassicanapin and sinigrin yield 3-methylsulfinylpropyl-ITC, 3-butenyl-ITC, 4-methylsulfinylbutyl-ITC (sulforaphane), 4-pentenyl-ITC and 2-propenyl-ITC (allyl-ITC), respectively.

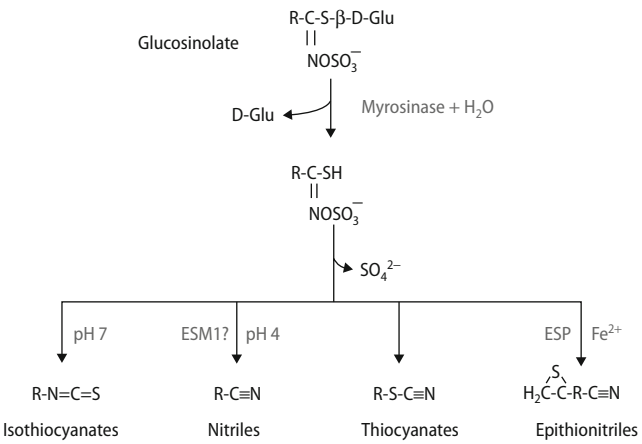

Fig. 2 Hydrolysis of Glucosinolates. At high or neutral pH the formation of isothiocyanates is favoured while at low pH the formation of nitriles is favoured. Epithiospecifier protein in the presence of Fe^{2+} ions interacts with myrosinase to promote the transfer of the sulfur to the alkenyl group from the S-Glucose of the terminally unsaturated Glucosinolate [6]

At low pH, the thiohydroximate-*O*-sulfates formed by myrosinase from glucosinolates with a side chain containing a double bond (e.g. sinigrin, gluconapin and glucobrassicanapin) may, in the presence of an epithiospecifier protein (ESP) and ferrous ions, give rise to a cyano-epithioalkane [102]. In this case, ESP interacts with myrosinase to promote sulfur transfer from the *S*-glycosyl unit to the alkenyl chain derived from the amino acid part of the aglycone [39]. Thus, at pH 4 and in the presence of Fe^{2+} ions, myrosinase and ESP convert sinigrin to 1-cyano-2,3-epithiopropane [68]; Fig. 3 shows hydrolysis products produced from sinigrin. Gluconapin can similarly be converted by the combined actions of myrosinase and ESP to 1-cyano-3,4-epithiobutane [17, 63]. Likewise, glucobrassicanapin can be hydrolysed to 1-cyano-4, 5-epithiopentane [17]. Progoitrin, a (2R)-hydroxy-3-butenyl glucosinolate, is converted in the presence of myrosinase, ESP and Fe^{2+} ions to an epithionitrile [76]. In the case of epi-progoitrin [(2S)-hydroxy-3-butenyl glucosinolate], it can be hydrolysed by myrosinase to crambene (1-cyano-2-hydroxy-3-butene) [24, 40]. Two cDNAs for ESP have been cloned from Arabidopsis and broccoli, and the purified proteins characterized following their heterologous expression in E. coli [82, 139]. In Arabidopsis, an epithiospecifier modifier (ESM) gene has been reported that inhibits formation of the epithionitrile and favours production of nitrile [144].

If the aglycone generated by myrosinase is from a glucosinolate with a side chain lacking a double bond, the sulfur atom may be lost and a nitrile formed [69,

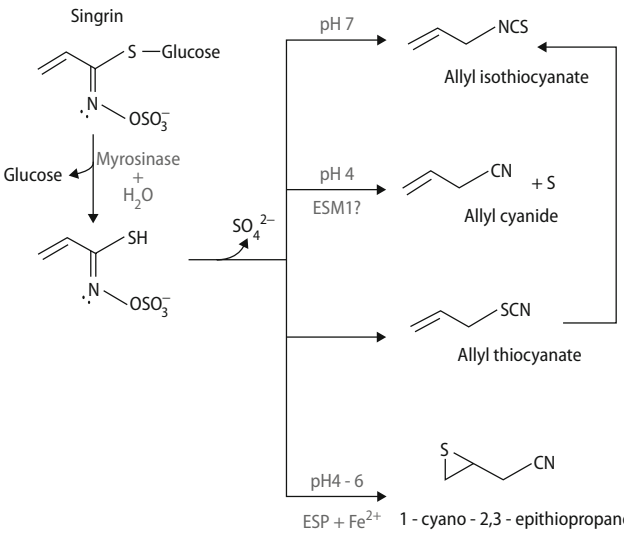

Fig. 3 Hydrolysis of Sinigrin. Following damage to the plant tissue, the glucosinolate sinigrin is hydrolysed by myrosinase resulting in the formation of four distinct compounds

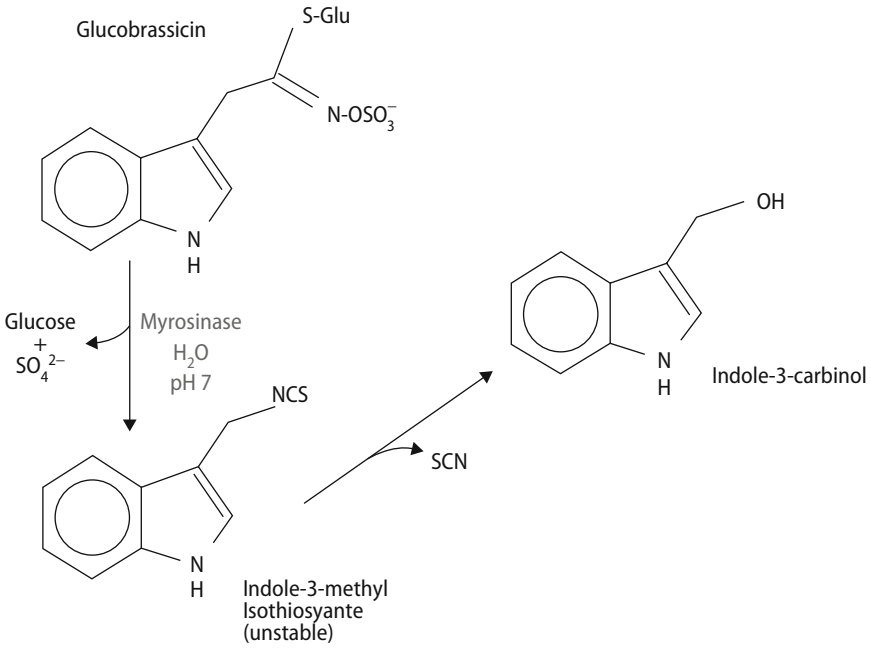

Fig. 4 Production of Indoles from Glucosinolates. At neutral pH the hydrolysis of glucobrassican by myrosinase leads to the formation of an unstable isothiocyanate intermediate that degrades to form Indole-3-carbinol and a thiocyanate ion

80, 90]. This reaction may involve ESP, and is diminished by heating [81]. A few glucosinolates produce thiocyanates though the mechanism involved is unclear [5, 6]. Upon hydrolysis by myrosinase, those aglycones from glucosinolates that contain *β*-hydroxylated side-chains form oxazolidine-2-thiones, as a consequence of spontaneous cyclization. Examples of these include progoitrin, glucoconringiin and gluconapoleiferin [55].

Production of indoles from glucosinolates

The indolyl glucosinolates glucobrassicin and neoglucobrassicin are synthesised by the plant from tryptophan. The best studied of these is glucobrassicin. At neutral pH, hydrolysis of glucobrassicin by myrosinase does not generate an ITC, but rather gives rise to indole-3-carbinol and a thiocyanate ion (Fig. 4); this reaction probably proceeds through a Lossen rearrangement generating an unstable ITC intermediate [83]. At acidic pH, hydrolysis of glucobrassicin yields indole-3-acetonitrile, hydrogen sulfide and elemental sulfur [83]. Formation of indole-3-acetonitrile requires both myrosinase and ESP [9]. In the acidic environment of the stomach, indole-3-carbinol condenses to form various compounds including indolo[3,2-*b*]carbazole and 3,3′-diindolylmethane, both of which have potent pharmacological effects [7, 25]. It can also combine with ascorbic acid to form ascorbigen [105] (Fig. 5).

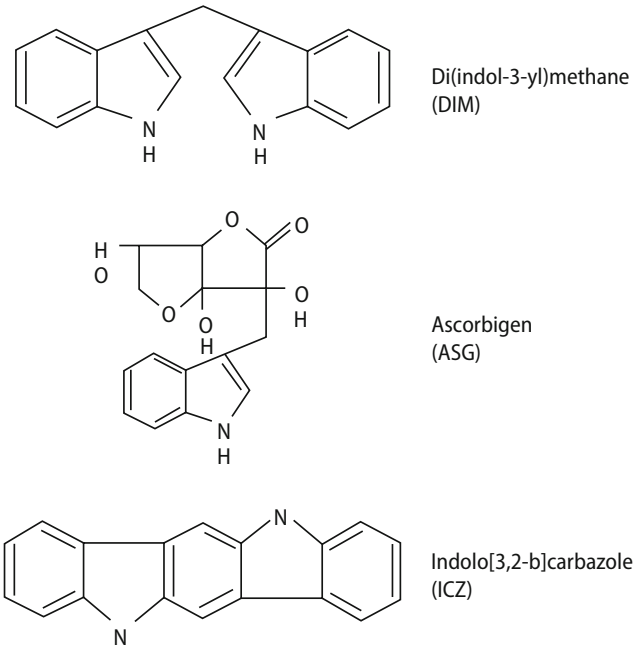

Fig. 5 Structures of indoles produced from Glucosinolates - 3,3′-Diindolylmethane (DIM), Indolo[3,2-*b*]carbazole (ICZ) and Ascorbigen (ASG)

Chemopreventive mechanisms stimulated by glucosinolate hydrolysis products

In view of the diverse spectrum of chemicals generated from glucosinolates by the actions of myrosinase, ESP and ESM, it is not surprising that a number of

distinct cancer chemopreventive mechanisms have been proposed to account for the putative anti-cancer properties of cruciferous vegetables. These include induction of antioxidant and detoxification genes through activation of Nrf2 (NF-E2 related factor 2) and AhR (arylhydrocarbon receptor), inhibition of pro-inflammatory reactions by repression of NF-κB (nuclear factor-κB), inhibition of cytochrome P450 (CYP) enzyme activity, inhibition of histone deacetylase, and stimulation of cell cycle arrest and apoptosis. There is a dose-dependency in these responses: generally, induction of cytoprotective genes and inhibition of CYP activity occurs at relatively low concentrations of phytochemical, whereas activation of cell cycle arrest and apoptosis occurs at higher levels of phytochemical [7, 62]. A major problem exists in interpreting experiments utilizing vegetable extracts because of their inherent variable composition and thus uncertainty about attributing biological effects to specific phytochemicals. It is also apparent that uncertainties exist about the bioavailability of many glucosinolate breakdown products that further complicates interpretation of in vitro data [48]. A challenge in evaluating the literature arises from the emphasis placed on certain glucosinolate breakdown products and the dearth of data relating to others. Thus, there is a relative abundance of information about isothiocyanates and indoles, when compared with the data about thiocyanates, nitriles, cyano-epithioalkanes, and oxazolidine-2-thiones. Considerable evidence has, for example, been presented that the ITC sulforaphane can inhibit experimental chemical carcinogenesis in animal models at various sites including mammary gland [142], stomach [33] and skin [133]. Sulforaphane can also protect against familial adenomatous polyposis in the intestine of $Apc^{Min/+}$ mice [113]. Also, broccoli extracts enriched with sulforaphane can prevent UV-light initiated skin carcinogenesis [29], and sulforaphane activates cell defences against UV radiation [121]. Comparatively little testing of the chemopreventive properties of other glucosinolate-derived phytochemicals in animal models has been reported, though there is a body of literature that indicates indoles can exert promoting effects in experimental chemical carcinogenesis (see ref [55] for a review).

Induction of gene expression mediated by Nrf2

Isothiocyanates increase the expression of antioxidant and detoxication proteins, such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), both in vivo and in vitro [96, 147]. Agents such as ITC that increase GST and NQO1

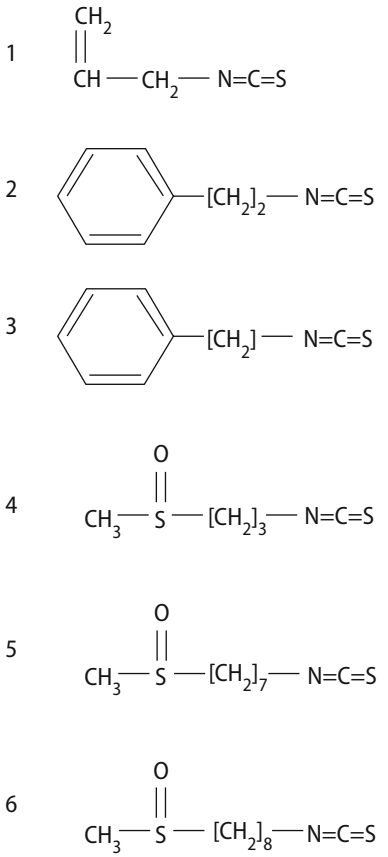

Fig. 6 Structures of isothiocyanates which induce NQO1: **1-** Allyl ITC, **2-** Phenethyl ITC, **3-** Benzyl ITC, **4-** 3-methylsulfinylpropyl ITC, **5-** 7-methylsulfinylheptyl ITC, **6-** 8-methylsulfinyloctyl ITC

enzymes without increasing arylhydrocarbon hydroxylase activity, catalysed by the phase I drug-metabolising enzyme CYP 1A1, are sometimes called mono-functional inducers [106]. Sulforaphane, 3-methylsulfinylpropyl-ITC, allyl-ITC, 7-methylsulfinylheptyl-ITC, 8-methylsulfinyloctyl-ITC, benzyl-ITC and phenethyl-ITC, induce NQO1 in the mouse Hepa-1c1c7 hepatoma cell line [27, 146] (Fig. 6). Many of these compounds induce GST P1-1 in the rat liver RL34 cells [91]. The mouse *nqo1* gene contains a functional antioxidant response element (ARE, minimal enhancer 5′-$^{A}/_{G}$TGA$^{C}/_{G}$NNNGC$^{A}/_{G}$-3′), sometimes called an electrophile response element (EpRE), in its 5′-upstream region [100], as does the rat *GSTP1* gene (in which it was originally called glutathione transferase P enhancer I, GPEI) [44]. The induction of these two genes by ITCs is mediated by the Nrf2 bZIP (basic-region leucine zipper) transcription factor that is recruited to the ARE as a heterodimer with a small Maf protein, MafF, MafG or MafK [100]. As far as is known, all genes that are induced by ITCs contain an ARE in their promoters and are regulated by Nrf2. Examination of $nrf2^{-/-}$ and wild-type mice, suggests

AREs are present in the promoters of at least 100 genes. The battery of genes regulated by Nrf2 includes those for antioxidant proteins, drug-metabolising enzymes, drug efflux pumps, heat shock proteins, and α and β subunits of the 26S proteasome [54, 67, 84, 124]. Specifically, some of the most inducible genes in rodent and human cells are those encoding aldo-keto reductase (AKR), carboxyl esterase, ferritin, glutamate cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits, GST, heme oxygenase 1, NQO1, metallothionein, microsomal epoxide hydrolase, multidrug resistance-associated protein, thioredoxin, thioredoxin reductase and UDP-glucuronosyl transferase [26, 54, 67, 84, 124]. Many of these genes are induced by sulforaphane in vivo in an Nrf2-dependent fashion in the stomach, small intestine and liver of rodents [34, 51, 54, 84, 124]. Importantly, feeding broccoli seed to mice increased the levels of GCLC, GST and NQO1 in the gastrointestinal tract in an Nrf2-dependent fashion [87].

It is thought that ITCs possess the ability to induce ARE-driven gene expression because they are thiol-active [30]. Through this characteristic, it is highly probable that dietary ITCs modify cysteine residues in many proteins. They also react with glutathione [140] and therefore presumably produce redox stress by altering the intracellular GSH:GSSG ratio. Most significantly in terms of eliciting an adaptive response to such stress, sulforaphane forms adducts with cysteines in Keap1 (Kelch-like ECH-associated protein 1) [31, 49], a Cullin-3:Rbx1 E3 ubiquitin ligase substrate adaptor protein [64, 143], and carbamylation of certain critical residues (most frequently thought to be Cys-273 and Cys-288, though there is not a consensus view on this point, c.f. [31, 49, 126, 141]) in the central intervening region (IVR) of Keap1 leads to failure of the Cullin-3:Rbx1/Keap1 complex to ubiquitylate Nrf2. Within the cell, Keap1 is a dimeric zinc-containing protein [28] and binds Nrf2 through both of its C-terminal Kelch-repeat domains interacting simultaneously with a low-affinity DLG motif and a high-affinity ETGE motif in the N-terminal Neh2 domain of the bZIP factor [86]; see Fig. 7 for a cartoon of the two-site interaction between the Keap1 dimer and Nrf2. As a consequence of this two-site interaction, the lysine acceptor sites in Nrf2 that lie between the DLG and ETGE motifs are immobilized and presented to Cullin-3:Rbx1 in an orientation that allows their ubiquitylation. Under normal homeostatic conditions, this process is highly efficient and results in rapid proteasomal degradation of the transcription factor. It appears most likely that modification of Keap1 by sulforaphane causes a conformational change in the substrate adaptor that prevents it from binding both the low-affinity DLG and high-affinity ETGE motifs in Nrf2. Through modification by ITC, it seems likely the Cullin-3:Rbx1/Keap1/Nrf2 complex becomes "stalled", with Nrf2 only being bound to one of the Kelch-repeat domains via its high-affinity ETGE motif [86]. This will probably result in Keap1 becoming saturated, thereby allowing newly translated Nrf2 to avoid

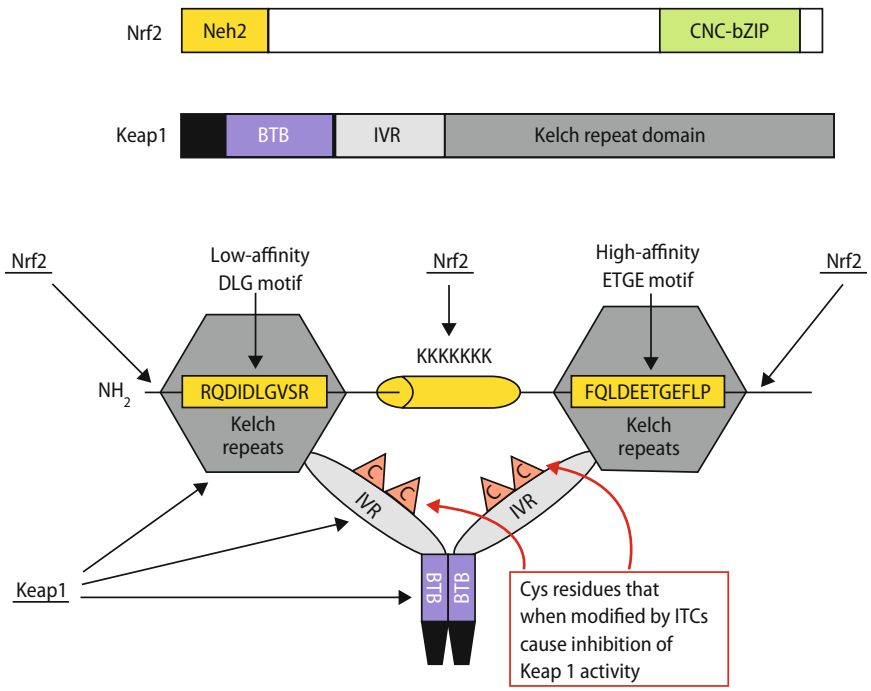

Fig. 7 The domain structures of Nrf2 and Keap1, and the complex formed between the two proteins. The cartoon shows interaction between the two Kelch-repeat domains found in the dimeric Keap1 protein with the low-affinity DLG motif and the high-affinity ETGE motif found in the Neh2 domain of Nrf2 [86]. The seven lysine residues located between the DLG and ETGE motifs in the Neh2 domain that serve as ubiquitin acceptor sites are shown. The Cys residues in the IVR of Keap1 that are modified by ITCs are also depicted

capture by Keap1 and accumulate within the cell. Consistent with this view, exposure of RL34 cells or HepG2 cells to sulforaphane causes stabilisation and rapid accumulation of Nrf2 [57, 85]. Surprisingly, allyl-ITC does not increase Nrf2 stability [57] and therefore other factors may be involved in enzyme induction by these phytochemicals. Treatment of cells with benzyl-ITC causes a rapid increase in the level of reactive oxygen species, and this could also contribute to gene induction [97].

Up-regulation of thioredoxin and thioredoxin reductase (TrxR1) by sulforaphane is controlled at several levels [2, 145]. Induction of mRNA for TrxR1 by sulforaphane occurs through an ARE in its gene promoter, presumably mediated by Nrf2. However, as TrxR1 is a seleno-protein, the consequence of increased gene transcription can be augmented at the translational level by sodium selenite, and other forms of selenium, through providing an adequate supply of SeCys for incorporation into protein and also by delaying degradation of TrxR1 mRNA [145].

It has been found that administration of cranbene to Fischer 344 rats causes an elevation in hepatic GST and NQO1 enzyme activities, but not CYP1A1, suggesting it is a mono-functional inducer [78]. By comparison with sulforaphane, cranbene was found to be an approximately equally potent inducer in the rat. However, it was not a particularly effective inducer of NQO1 activity in Hepa-1c1c7 cells suggesting that the relatively high potency of induction observed in vivo is due to bio-transformation of cranbene to a thiol-active metabolite [61]. The identity of this metabolite is not known.

The indole-containing glucobrassicin breakdown products can activate gene expression by several mechanisms. Indole-3-carbinol is a modest inducer of Nrf2-dependent ARE-driven gene expression in the liver and small intestine of mice [12, 84]. Although indole-3-acetonitrile has not been studied in Nrf2 knockout mice, it is a good inducer of GST enzyme activity in wild-type mouse liver and small intestine [45], a fact that infers it works through Nrf2.

The activity of the Nrf2 transcription factor can be inhibited by retinoic acid through a protein–protein interaction between Nrf2 and retinoic acid receptor alpha that prevents the bZIP protein from binding to the ARE [127]. Similarly, the nuclear orphan receptor estrogen-related receptor beta also negatively regulates Nrf2 [149]. At present it is not known whether dietary retinoids and their precursors, or dietary estrogens, can block the benefits of ITCs in vivo, but this will be an important point to clarify as the presence of high levels of retinoic acid could represent a confounding factor in clinical intervention studies.

Induction of gene expression mediated by AhR

The major effect indole-3-carbinol has on gene expression arises because it can condense in acid conditions to form indolo[3,2-*b*]carbazole and 3,3′-diindolylmethane. Both these condensation products induce *CYP1A1* genes via the xenobiotic response element (XRE, 5′-T$^{A}/_{T}$GCGTG$^{A}/_{C}$-3′) in their promoter regions because they are ligands for the aryl-hydrocarbon receptor (AhR), a basic helix-loop-helix transcription factor. Examination of the dose of indole required to double XRE-driven reporter gene expression showed that indolo[3,2-*b*]carbazole is a much more potent inducing agent than 3,3′-diindolylmethane, indole-3-carbinol or ascorbigen [4, 7]. Amongst genes for drug-metabolising enzymes, mouse, rat and human *CYP1A1* are prototypic XRE-regulated genes. This cytochrome has *O*-deethylase activity towards ethoxyresorufin, and there is abundant evidence from enzyme assays, western or northern blotting that CYP1A1 is inducible by indolo[3,2-*b*]carbazole and 3,3′-diindolylmethane [55]. The promoters of other genes including rat and human *NQO1*, rat *ALDH-3*, rat *GSTA2*, rat *UGT1A1*, and rat *UGT1A6* contain an XRE, as does the mouse *BAX* gene, and it is therefore anticipated that activation of AhR by glucobrassicin-derived indoles will influence significantly the metabolism of xenobiotics; for a review, see [99] and for a more recent report of gene expression profiling to identify AhR target genes, see [125].

Significantly, the mouse *nrf2* gene promoter also contains an XRE, and AhR ligands such as dioxin produce an increased production of Nrf2 mRNA [88] (Fig. 8). As Nr2 is regulated primarily at the level of protein stability [85], the increase in mRNA will not in itself cause an increase in ARE-driven gene expression unless the rate of translation of Nrf2 is sufficiently high to saturate the Cullin-3:Rbx1/Keap1 complex. However, the combination of an AhR ligand, such as indolo[3,2-*b*]carbazole, plus a redox stressor, such as an ITC, should result in a more marked increase in Nrf2 protein than occurs through redox stress alone; Fig. 9 shows how indoles and ITCs could act synergistically. Whether indolo[3,2-*b*]carbazole and 3,3′-diindolylmethane can increase both Nrf2 mRNA levels and the stability of Nrf2 protein is not known, but unless these phytochemicals are metabolised by CYPs, and thereby generate reactive oxygen species, this seems unlikely [98].

In addition to up-regulation of CYP1A1 by indoles, CYP1B1 and CYP19 are inducible [112], as are the drug-metabolising enzymes AKR, GST T1-1, sulfotransferase and UGT1 [71]. Furthermore, 3,3′-diindolylmethane can induce the transcription

Species	Gene	Element	5′-USR	enhancer	3′-USR	3′ nucleotide
mouse	*nrf2*	XREL1	gtttg	cAGCGTGg	actca	-706
mouse	*nqo1*	XRE	tcccc	TAGCGTGC	aaagg	-374
rat	*NQO1*	XRE	tcccc	TTGCGTGC	aaagg	-360
human	*NQO1*	XRE	attac	agGCGTGA	gcacc	-730
rat	*ALDH-3*	XRE	gccgc	cTGCGTGA	ctgca	-376
mouse	*cyp1a1*	DRE1	gaggc	TAGCGTGC	gtaag	-903
mouse	*cyp1a1*	DRE2	ccagc	TAGCGTGA	cagca	-1052
mouse	*cyp1a1*	DRE3	cggag	TTGCGTGA	gaaga	-976
rat	*CYP1A1*	XRE1	cggag	TTGCGTGA	gaaga	-1020
rat	*CYP1A1*	XRE2	ccagc	TAGCGTGA	cagca	-1079
human	*CYP1A1*	XRE1	aggcg	TTGCGTGA	gaagg	-991
human	*CYP1A1*	XRE2	ccccc	TcGCGTGA	ctgcg	-1048
rat	*GSTA2*	XRE	gcatg	TTGCGTGC	atccc	-893
rat	*UGT1A1*	XRE	agaat	gTGCGTGA	caagg	-123
human	*UGT1A6*	XRE	agaac	TcGCGTGC	agcag	-1492
XRE 'core' consensus.....				TAGCGTGA T C		

Fig. 8 Presence of an XRE in the promoter of the *nrf2* gene. The sequence data for the XRE in the promoter of *nrf2* are taken from ref [88]. The other XRE-containing sequences are taken from ref [99]

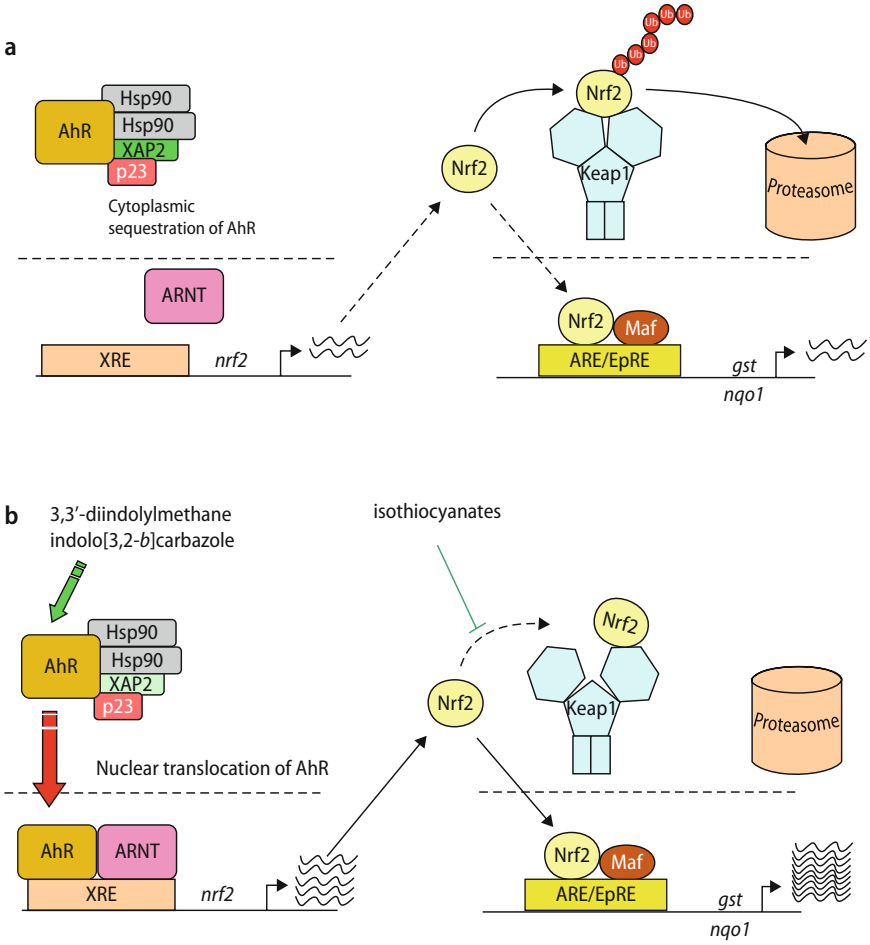

Fig. 9 Cross-talk between the AhR-XRE pathway and the Nrf2-ARE pathway. **a** Under normal homeostatic conditions, Nrf2 is primarily ubiquitylated and degraded through the 26S proteasome, with only a relatively small fraction of the total translated protein being recruited to the promoters of ARE-driven genes. **b** The combined effect of i) indoles inducing transcription of the *nrf2* gene through AhR and ii) isothiocyanates preventing ubiquitylation of the increased load of translated Nrf2 protein, by inhibition of Keap1, could theoretically result in hyper-induction of ARE-driven genes

factors ATF3, c-Jun and NF-IL6 as well as genes involved in cell growth such as growth arrest and DNA damage (GADD) GADD34, GADD45 and GADD153 [1, 11]. Also, p21 is induced by indole-3-carbinol [19]. It is not however clear whether the genes for ATF3, c-Jun, and NF-IL6, and the various GADD genes, are regulated through XREs in an AhR-dependent fashion.

It has been argued that induction of CYP1A1 by indoles is potentially deleterious to the cell because the cytochrome can activate polycyclic aromatic hydrocarbons to ultimate carcinogens. This viewpoint is probably an oversimplification and does not take into account the multiple changes in gene expression that indoles affect. Bonnesen et al. [7] have reported that treatment of human colon LS-174 cells with indolo[3,2-*b*]carbazole before exposure to benzo[*a*]-pyrene provides a small measure of protection against DNA damage as measured by a Comet assay. Most importantly, prior treatment of the LS-174 cells with both indolo[3,2-*b*]carbazole and sulforaphane before exposure to benzo[*a*]pyrene was found to confer substantial protection against genotoxicity, and this protection was greater than was achieved by either phytochemical alone [7].

It is interesting to note that some cross-talk appears to exist between the Nrf2 and AhR pathways insofar as AhR requires the presence of Nrf2 in order to induce the expression of NQO1 by dioxin or 3-methylcholanthrene [75, 101]. As mentioned above, the promoter of mouse *nrf2* contains several XREs [88] and the promoter of mouse *ahr* contains an ARE [114]. Thus AhR regulates Nrf2 mRNA levels, and Nrf2 regulates AhR mRNA levels. It remains to be tested whether combined exposure of cells to AhR ligands such as indolo[3,2-*b*]carbazole along with stabilizers of Nrf2 protein such as ITCs will have synergistic effects on both ARE-driven gene expression and XRE-driven gene expression.

Inhibition of pro-tumourigenic processes by glucosinolate breakdown products

Inflammation is a well-recognized risk factor in carcinogenesis. Isothiocyanates possess anti-inflammatory activity through inhibiting NF-κB [47, 119]. The ability of sulforaphane and phenethyl-ITC to inhibit the transcriptional activity of NF-κB is a consequence of the phytochemicals antagonising phosphorylation of IκB, the inhibitor of NF-κB, which is carried out by IKK [134]. Inhibition of NF-κB prevents transcriptional activation of genes such as cyclin D1, VEGF, Bcl-X_L, COX2 and MMP-9. Although these gene products influence various biological processes, inhibition of NF-κB generally appears to make cells more sensitive to apoptosis. Interestingly, circumin and cyclopentenone prostaglandins, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, also antagonize NF-κB by inhibiting IKK [16, 103]. As circumin and cyclopentenone prostaglandins contain an α,β-unsaturated carbonyl group they are, like ITCs, thiol-reactive. It has been proposed that 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inactivates IKK by modifying Cys-179 [111], and it would be interesting to know if ITCs modify the same cysteine residue.

Certain isothiocyanates can block the activation of several carcinogens to their ultimate carcinogenic forms. Tumorigenesis caused by the carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N-nitrosobis-(2-oxopropyl)amine can be prevented by phenethyl-ITC and this involves inhibition of activation of pro-carcinogens by CYP isoenzymes [46, 95]. Inhibition of CYP can also be achieved by sulforaphane [18, 77]. In the case of the nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, the ITCs with highest lipophilicity and low reactivity of their NCS group had the greatest ability to inhibit lung tumourigenesis [58].

Inhibition of histone deacetylase by isothiocyanates

The isothiocyante sulforaphane has been shown to inhibit histone deacetylase (HDAC) [93, 94]. This function is likely to alter gene expression substantially. It may also have profound implications for cell fate as a change in the balance between histone acetyl transferase (HAT) and HDAC could alter tumourigenesis. Indeed, recognition of this possibility has lead to considerable recent interest in the ability of HDAC inhibitors to act as both chemopreventive and chemotherapeutic agents.

Within the cell, DNA is tightly coiled around an octamer of histone proteins in a structure known as a nucleosome, the basic structural unit of chromatin. Each of the histone proteins contains an evolutionary conserved amino tail protruding from the nucleosome, which can determine the accessibility of the DNA to transcription factors. The tail is also subject to many post-translational modifications including acetylation. The addition of an acetyl group to the histone tail results in a conformational change which enables the tail to move away from the DNA allowing transcription factors access to interact with the DNA. Conversely, removal of acetyl groups causes the tail to wrap tightly around the DNA thereby preventing interaction with the transcription machinery. The addition and removal of the acetyl groups is carried out by HAT and HDAC, respectively. In pre-cancerous and cancerous cells, tumour suppressor genes are associated with deacetylated histones resulting in the inactivation of these genes. Inhibition of HDAC may prevent the removal of acetyl moieties from histones, thus allowing transcription of the tumour suppressor genes.

Sulforaphane has been shown to diminish HDAC activity with a concomitant rise in histone acetylation

in prostate cancer cells [93], human embryonic kidney cells [94] and human colorectal cancer cells [94]. The link between inhibition of HDAC activity and the resultant increase in transcription of tumour suppressor genes has been reported for p21 [92, 93], p53 [38] and Bax [92, 93]. Equally, mammalian HDAC is capable of down-regulating p53 function, by deactylation of the *p53* gene, resulting in a reduction in its transcriptional activity. In addition, sulforaphane has been reported to cause a G_2/M phase delay with an increase in apoptotic cell fraction in a time and dose dependent fashion [60].

The ability of sulforaphane, along with other dietary HDAC inhibitors such as diallyl disulfide [22], to alter chromatin structure is likely to be of considerable biological significance but its contribution to chemoprevention is poorly understood.

Stimulation of cell cycle arrest and apoptosis by isothiocyanates

Many of the naturally occurring ITCs can suppress the growth of cultured tumour cells by modulating multiple targets that influence cell cycle arrest, apoptosis and differentiation [148]. However, the majority of the studies into mechanisms by which this class of chemical inhibit cell growth have focussed on sulforaphane and phenethyl-ITC. For example, $Apc^{Min/+}$ mice treated with sulforaphane at either 300 or 600 ppm in their diet have been reported to develop fewer and smaller polyps in their small intestine than $Apc^{Min/+}$ mice on a control diet; this was associated with a higher level of apoptosis and lower cell proliferation in animals on the ITC-containing diet [52].

In human PC-3 prostate cancer cells, treatment with sulforaphane or phenethyl-ITC causes an arrest in G_2/M phase of the cell cycle that is associated with a decrease in levels of cyclin B1 and cell division cycle (Cdc) 25B and Cdc25C proteins [115, 130]. The loss of Cdc25C was reported to be due to proteasomal activity, and was accompanied by its translocation from the nucleus to the cytoplasm [115]. Relocation of Cdc25C was controlled by its phosphorylation at Ser-216, mediated through activation of checkpoint kinase 2 (Chk2). Cell proliferation by ITCs may also be achieved by distrupting cytoskeletal structure and tubulin polymerisation [56, 117].

Administration of ITCs to cells at growth suppressive concentrations results in the rapid generation of reactive oxygen species (ROS), within 1 h of exposure, which appears to be necessary for cell death [115, 116]. The generation of ROS by ITCs is accompanied by depletion of intracellular GSH and is achieved through the rapid export of ITC-glutathione and ITC-cysteinylglycine conjugates via MRP1 and Pgp-1 efflux pumps [10]. Consistent with the view that production of ROS is necessary for apoptosis, overexpression of catalase suppresses ITC-initiated apoptosis, as does pre-treatment with *N*-acetylcysteine [115]. Furthermore, addition of GSH subsequent to ITC treatment can block apoptosis [62, 136]. Treatment with ITCs leads to a loss of mitochondrial membrane potential and release of cytochrome c from mitochondria [123]. There is evidence that ITCs can activate both the intrinsic and extrinsic caspase cascades, though this may be cell specific. For example, in PC-3 cells, sulforaphane can increase Fas protein levels and activate caspase-8 whilst simultaneously targeting mitochondria and activating caspase-9 [115]. In human bladder cancer UM-UC-3 cells, benzyl-ITC and phenethyl-ITC are more effective in activating caspase-9 than caspase-8 [122]. By contrast, in human leukaemia HL60 cells, caspase-8 plays a major role in apoptosis stimulated by phenethyl-ITC [135]. The levels of pro-apoptotic proteins Bak and Bax, which neutralize the antiapoptotic effects of Bcl-2, are increased by phenethyl-ITC and sulforaphane in PC-3 prostate cancer cells, and this may lead to induction of Apaf-1 [115, 130, 132]. Furthermore, the pro-apoptotic proteins Bok and Bim EL are also induced by ITCs, and this is thought to amplify the effects of Bak and Bax [115]. Besides increasing the levels of these pro-apoptotic proteins, ITCs down-regulate the anti-apoptotic proteins Mcl-1 and Bcl-x_L, though the effect is cell-specific [132]; for a review of regulation of apoptotic pathways by BCL-2 family proteins see [138]. Various ITCs have been shown to activate c-Jun N-terminal kinase (JNK) [13, 137], and this is mediated by extracellular signal-regulated kinases, ERK1/2 [131]. The use of inhibitors has indicated that JNK is essential for phenethyl-ITC to cause cytochrome c release and caspase-3 activation in human HT-29 colon adenocarcinoma cells [53]. However, the mechanism by which JNK activates caspases remains unclear.

Stimulation of cell cycle arrest and apoptosis by indoles

Treatment of human MCF-7 breast cancer cells with 100 μM indole-3-carbinol inhibits proliferation through affecting a G_1 cell cycle arrest [20]. This may in part be due to 3,3′-diindolylmethane rather than indole-3-carbinol as significant quantities of the indole spontaneously condense to the dimer in culture conditions [120]. Cell cycle arrest at G_1 occurs as a consequence of indole-3-carbinol inhibiting both

cyclin-dependent kinase (CDK) 2 and CDK6. In the case of CDK6, expression of the gene is reduced because indole-3-carbinol attenuates recruitment of the Sp1 transcription factor to the *CDK6* promoter [21]. Furthermore, in HaCaT keratinocytes, treatment with 400 μM indole-3-carbinol induces the CDK4/6 inhibitor $p15^{INK4b}$ mRNA and protein causing hypophosphorylation of Rb protein [79]. It therefore appears that cyclin D-CDK6 activity can be inhibited by dual mechanisms, though the concentrations of indole involved seem rather high. In the case of CDK2, indole-3-carbinol has been reported to decrease the kinase activity in MCF-7 cells and inhibit phosphorylation of Rb protein [11]. The reduction in CDK2 activity is attributed to a selective alteration in the size of the complex in which it is contained, from an active form within a 90 kDa complex to a lower activity form within a 200 kDa complex [41]; the 90 and 200 kDa complexes include forms of cyclin E that differ in size, and the larger complex also contains an additional 75 kDa cyclin E immunoreactive protein. Furthermore, the reduction in CDK2 activity is accompanied by redistribution of the kinase in the 200 kDa complex from the nucleus to the cytoplasm, suggesting that indole-3-carbinol can influence the nucleocytoplasmic shuttling of the kinase [41].

In cells that contain wild-type p53, such as MCF-10A, treatment with 300 μM indole-3-carbinol or 30 μM 3,3′-diindolylmethane has been found to result in activation of the ATM signalling pathway, an increase in p53 protein levels, and induction of p21 [8]. These changes result in prevention of the CDK2-mediated G_1/S transition in the cell cycle [8].

Indole-3-carbinol, but not 3,3′-diindolylmethane, was found to inhibit expression of the androgen receptor in human lymph node carcinoma of prostate (LNCaP) cells as well as the probable downstream target gene prostate specific antigen [50]. It is possible that down-regulation of this receptor represents an antiproliferative mechanism in prostate cells.

Indoles can affect apoptosis in breast and prostate cancer cells. Treatment of PC-3 prostate cancer cells with 60 μM indole-3-carbinol inhibits the EGF-induced autophosphorylation of PI3K and Akt [14]. Thus, the Akt/PI3K cell survival pathway appears to be targeted by indole-3-carbinol. Also, nuclear translocation of NF-κB is inhibited by 3,3′-diindolylmethane through a reduction in phosphorylation of IκBα [107, 108].

In HCT-116 human colon cells, indole-3-carbinol can induce nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1), a TGF-β family member associated with pro-apoptotic activities [70]. This may also mediate the anti-tumour effects of indoles.

Concluding comments

It is becoming clear that glucosinolate breakdown products can influence the initiation and progression of carcinogenesis. They also appear to influence apoptotic responses to chemotherapeutic agents, such as tamoxifen [19]. A major impediment to our understanding of the chemopreventative mechanisms stimulated by glucosinolates is that relatively little is known about the biological effects of glucosinolate breakdown products other than isothiocyantes and the indole-containing derivatives. Specifically, there are little data about chemopreventative activities of thiocyanates, nitriles, cyano-epithioalkanes and oxazolidine-2-thiones. It is unclear whether formation of thiocyanates, nitriles, cyano-epithioalkanes and oxazolidine-2-thiones from glucosinolates, at the expense of forming isothiocyanates, is undesirable from a cancer chemoprevention perspective. It is unclear whether the activity of ESP, which reduces the formation of isothiocyanates from glucosinolates, is undesirable. If so, ESP should possibly be eliminated by genetic means from commercial crops. Furthermore, relatively little is known about the pharmacokinetic properties of glucosinolate breakdown products in the human, and without this information it is difficult to relate responses of cells in culture to certain concentrations of phytochemical to the in vivo situation. These are areas that warrant further examination.

Mammalian cells display marked dose responsiveness to phytochemicals: at low doses of phytochemical, cytoprotective adaptive responses are activated, whereas at higher doses cell cycle arrest and apoptosis occurs. It is presently unclear how these different types of response are co-ordinated by the cell and how decisions about whether adaptation, growth arrest or apoptosis is chosen as the appropriate response are determined. Identification of mechanisms that control such outcomes will be useful.

Acknowledgements We thank Prof Björn Åkesson for critically reading this paper. This article was written as a part of the research integration in the work-package "Mechanisms of modulation of cancer by dietary factors" in the NoE Environmental Cancer risk, Nutrition and Individual Susceptibility (ECNIS, no. 513943; http://www.ecnis.org <http://www.ecnis.org>). We are grateful to the World Cancer Research Fund (2002/55) and the Association for International Cancer Research (04-088) for supporting some of our work described in this article. Conflict of interest: None of the authors has any financial conflict of interest. We have no commercial arrangement with companies that market glucosinolates nor do we act as consultants for companies working in the phytochemical area.

References

1. Auborn KJ, Fan S, Rosen EM, Goodwin L, Chandraskaren A, Williams DE, Chen D, Carter TH (2003) Indole-3-carbinol is a negative regulator of estrogen. J Nutr 133:2470S–2475S
2. Bacon JR, Plumb GW, Howie AF, Beckett GJ, Wang W, Bao Y (2007) Dual action of sulforaphane in the regulation of thioredoxin reductase and thioredoxin in human HepG2 and Caco-2 cells. J Agric Food Chem 55:1170–1176
3. Bak S, Olsen CE, Petersen BL, Moller BL, Halkier BA (1999) Metabolic engineering of p-hydroxybenzylglucosinolate in Arabidopsis by expression of the cyanogenic CYP79A1 from Sorghum bicolor. Plant J 20:663–671
4. Bjeldanes LF, Kim J-Y, Grose KR, Bartholomew JC, Bradfield CA (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc Natl Acad Sci USA 88:9543–9547
5. Bones AM, Rossiter JT (1996) The myrosinase-glucosinolate system, its organisation and biochemistry. Phys Plant 97:194–208
6. Bones AM, Rossiter JT (2006) The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 67:1053–1067
7. Bonnesen C, Eggleston IM, Hayes JD (2001) Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. Cancer Res 61:6120–6130
8. Brew CT, Aronchik I, Hsu JC, Sheen J-H, Dickson RB, Bjeldanes LF, Firestone GL (2006) Indole-3-carbinol activates the ATM signalling pathway independent of DNA damage to stabilize p53 and induce G1 arrest of human mammary epithelial cells. Int J Cancer 118:857–868
9. Burow M, Zhang ZY, Ober JA, Lambrix VM, Wittstock U, Gershenzon J, Kliebenstein DJ (2008) ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in Arabidopsis. Phytochemistry 69:663–671
10. Callaway EC, Zhang Y, Chew W, Chow HH (2004) Cellular accumulation of dietary anticarcinogenic isothiocyanates is followed by transporter-mediated export as dithiocarbamates. Cancer Lett 204:23–31
11. Carter TH, Liu K, Ralph W Jr, Chen D, Qi M, Fan S, Yuan F, Rosen EM, Auborn KJ (2002) Diindolylmethane alters gene expression in human keratinocytes in vitro. J Nutr 132:3314–3324
12. Chanas SA, Jiang Q, McMahon M, McWalter GK, McLellan LI, Elcombe CR, Henderson CJ, Wolf CR, Moffat GJ, Itoh K, Yamamoto M, Hayes JD (2002) Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. Biochem J 365:405–416
13. Chen YR, Wang W, Kong AN, Tan TH (1998) Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. J Biol Chem 273:1769–1775
14. Chinni SR, Sarkar FH(2002) Akt inactivation is a key event in indole-3-carbinol-induced apoptosis in PC-3 cells. Clin Cancer Res 8:1228–1236
15. Ciska E, Martyniak-Przybyszewska B, Kozlowska H (2000) Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J Agric Food Chem 48:2862–2867
16. Ciucci A, Gianferretti P, Piva R, Guyot T, Snape TJ, Roberts SM, Santoro MG (2006) Induction of apoptosis in estrogen receptor-negative breast cancer cells by natural and synthetic cyclopentenones: role of the IkappaB kinase/nuclear factor-kappaB pathway. Mol Pharmacol 70:1812–1821
17. Cole RA (1976) Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry 15:759–762
18. Conaway CC, Wang CX, Pittman B, Yang YM, Schwartz JE, Tian D, McIntee EJ, Hecht SS, Chung FL (2005) Phenethyl isothiocyanate and sulforaphane and their N-acetylcysteine conjugates inhibit malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice. Cancer Res 65:8548–8557
19. Cover CM, Hsieh SJ, Cram EJ, Hong C, Riby JE, Bjeldanes LF, Firestone GL (1999) Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells. Cancer Res 59:1244–1251
20. Cover CM, Hsieh SJ, Tran SH, Hallden G, Kim GS, Bjeldanes LF, Firestone GL (1998) Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling. J Biol Chem 273:3838–3847
21. Cram EJ, Liu BD, Bjeldanes LF, Firestone GL (2001) Indole-3-carbinol inhibits CDK6 expression in human MCF-7 breast cancer cells by disrupting Sp1 transcription factor interactions with a composite element in the CDK6 gene promoter. J Biol Chem 276:22332–22340
22. Dashwood RH, Myzak MC, Ho E (2006) Dietary HDAC inhibitors: time to rethink weak ligands in cancer chemoprevention? Carcinogenesis 27:344–349
23. Daxenbichler ME, Spencer GF, Carlson DG, Rose GB, Brinker AM, Powell RG (1991) Glucosinolate composition of seeds from 297 species of wild plants. Phytochemistry 30:2623–2638
24. Daxenbichler ME, VanEtten CH, Wolff IA (1968) Diastereomeric episulfides from *epi*-progoitrin upon autolysis of crambe seed meal. Phytochemistry 7:989–996
25. De Kruif CA, Marsman JW, Venekamp JC, Falke HE, Noordhoek J, Blaauboer BJ, Wortelboer HM (1991) Structure elucidation of acid reaction products of indole-3-carbinol: detection in vivo and enzyme induction in vitro. Chem Biol Interact 80:303–315
26. Devling TW, Lindsay CD, McLellan LI, McMahon M, Hayes JD (2005) Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. Proc Natl Acad Sci USA 102:7280–7285A
27. Dinkova-Kostova AT, Fahey JW, Talalay P (2004) Chemical structures of inducers of nicotinamide quinone oxidoreductase 1 (NQO1). Meth Enzymol 382:423–448
28. Dinkova-Kostova AT, Holtzclaw WD, Wakabayashi N (2005) Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein. Biochemistry 44:6889–6899
29. Dinkova-Kostova AT, Jenkins SN, Fahey JW, Ye L, Wehage SL, Liby KT, Stephenson KK, Wade KL, Talalay P (2006) Protection against UV-light-induced skin carcinogenesis in SKH-1 high-risk mice by sulforaphane-containing broccoli sprout extracts. Cancer Lett 240:243–252
30. Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P (2001) Potency of Michael reaction acceptors as inducers of enzymes that protect

against carcinogenesis depends on their reactivity with sulfhydryl groups. Proc Natl Acad Sci USA 98:3404–3409
31. Eggler AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD (2005) Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc Natl Acad Sci USA 102:10070–10075
32. Elfoul L, Rabot S, Khelifa N, Quinsac A, Duguay A, Rimbault A (2001) Formation of allyl isothiocyanate from sinigrin in the digestive tract of rats monoassociated with a human colonic strain of Bacteroides thetaiotaomicron. FEMS Microbiol Lett 197:99–103
33. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A (2002) Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci USA 99:7610–7615
34. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A (2002) Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci USA 99:7610–7615
35. Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51
36. Farnham MW, Wilson PE, Stephenson KK, Fahey JW (2004) Genetic and environmental effects on glucosinolate content and chemopreventive potency of broccoli. Plant Breed 123:60–65
37. Fenwick GR, Heaney RK, Mullin WR (1983) Glucosinolates and their breakdown products in food and food plants. CRC Crit Rev Food Sci Technol 18:123–201
38. Fimognari C, Nusse M, Berti F, Cantelli-Forti G, Hrelia P (2003) Sulforaphane modulates cell cycle and apoptosis in transformed and non-transformed human T lymphocytes. Ann NY Acad Sci 1010:393–398
39. Foo HL, GrØnning LM, Goodenough L, Bones AM, Danielsen B, Whiting DA, Rossiter JT (2000) Purification and characterisation of epithiospecifier protein from Brassica napus: enzymic intramolecular sulphur addition within alkenyl thiohydroximates derived from alkenyl glucosinolate hydrolysis. FEBS Lett 468:243–246
40. Galletti S, Bernadi R, Leoni O, Rollin P, Palmieri S (2001) Preparation and biological activity of four epiprogoitrin myrosinase-derived products. J Agric Food Chem 49:471–476
41. Garcia HH, Brar GA, Nguyen DH, Bjeldanes LF, Firestone GL (2005) Indole-3-carbinol (I3C) inhibits cyclin-dependent kinase-2 function in human breast cancer cells by regulating the size distribution, associated cyclin E forms, and subcellular localization of the CDK2 protein complex. J Biol Chem 280:8756–8764
42. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC (2003) A prospective study of cruciferous vegetables and prostate cancer. Cancer Epidemiol Biomarkers Prev 12:1403–1409
43. Graser G, Schneider B, Oldham NJ, Gershenzon J (2000) The methionine chain elongation pathway in the biosynthesis of glucosinolates in Eruca sativa (Brassicaceae). Arch Biochem Biophys 378:411–419
44. Hayes JD, Flanagan JU, Jowsey IR (2005) Glutathione transferases. Annu Rev Pharmacol Toxicol 45:51–88
45. Hayes JD, Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30:445–600
46. Hecht SS (2000) Inhibition of carcinogenesis by isothiocyanates. Drug Metab Rev 32:395–411
47. Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhäuser C (2001) Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J Biol Chem 276:32008–32015
48. Holst B, Williamson G (2004) A critical review of the bioavailability of glucosinolates and related compounds. Nat Prod Rep 21:425–447
49. Hong F, Freeman ML, Liebler DC (2005) Identification of sensor cysteines in human keap1 modified by the cancer chemopreventive agent sulforaphane. Chem Res Toxicol 18:1917–1926
50. Hsu JC, Zhang J, Dev A, Wing A, Bjeldanes LF, Firestone GL (2005) Indole-3-carbinol inhibition of androgen receptor expression and downregulation of androgen responsiveness in human prostate cancer cells. Carcinogenesis 26:1896–1904
51. Hu R, Hebbar V, Kim BR, Chen C, Winnik B, Buckley B, Soteropoulos P, Tolias P, Hart RP, Kong AN (2004) In vivo pharmacokinetics and regulation of gene expression profiles by isothiocyanate sulforaphane in the rat. J Pharmacol Exp Ther 310:263–271
52. Hu R, Khor TO, Shen G, Jeong WS, Hebbar V, Chen C, Xu C, Reddy B, Chada K, Kong AN (2006) Cancer chemoprevention of intestinal polyposis in $Apc^{Min/+}$ mice by sulforaphane, a natural product derived from cruciferous vegetable. Carcinogenesis 27:2038–2046
53. Hu R, Kim BR, Chen C, Hebbar V, Kong AN (2003) The roles of JNK and apoptotic signaling pathways in PEITC-mediated responses in human HT-29 colon adenocarcinoma cells. Carcinogenesis 24:1361–1367
54. Hu R, Xu C, Shen G, Jain MR, Khor TO, Gopalkrishnan A, Lin W, Reddy B, Chan JY, Kong AN (2006) Gene expression profiles induced by cancer chemopreventive isothiocyanate sulforaphane in the liver of C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice. Cancer Lett 243:170–192
55. International Agency for Research on Cancer Workgroup (2004) Cruciferous vegetables, isothiocyanates and indoles, Handbooks of cancer prevention, vol 9. IARC Press, Lyon
56. Jackson SJ, Singletary KW (2004) Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. Carcinogenesis 25:219–227
57. Jeong WS, Keum YS, Chen C, Jain MR, Shen G, Kim JH, Li W, Kong AN (2005) Differential expression and stability of endogenous nuclear factor E2-related factor 2 (Nrf2) by natural chemopreventive compounds in HepG2 human hepatoma cells. J Biochem Mol Biol 38:167–176
58. Jiao D, Eklind KI, Choi CI, Desai DH, Amin SG, Chung FL (1994) Structure-activity relationships of isothiocyanates as mechanism-based inhibitors of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. Cancer Res 54:4327–4333
59. Johnson IT (2002) Glucosinolates: bioavailability and importance to health. Int J Vitam Nutr Res 72:26–31
60. Juan LJ, Shia WJ, Chen MH, Yang WM, Seto E, Lin YS, Wu CW (2000) Histone deacetylases specifically down-regulate p53-dependent gene activation. J Biol Chem 275:20436–20443

61. Keck AS, Staack R, Jeffery EH (2002) The cruciferous nitrile crambene has bioactivity similar to sulforaphane when administered to Fischer 344 rats but is far less potent in cell culture. Nutr Cancer 42:233–240
62. Kim BR, Hu R, Keum YS, Hebbar V, Shen G, Nair SS, Kong AN (2003) Effects of glutathione on antioxidant response element-mediated gene expression and apoptosis elicited by sulforaphane. Cancer Res 63:7520–7525
63. Kirk JTO, MacDonald CG (1974) 1-Cyano-3,4-epithiobutane: a major product of glucosinolate hydrolysis in seeds from certain varieties of Brassica campestris. Phytochemistry 13:2611
64. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, Yamamoto M (2004) Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol 24:7130–7139
65. Krul C, Humblot C, Philippe C, Vermeulen M, van Nuenen M, Havenaar R, Rabot S (2002) Metabolism of sinigrin (2-propenyl glucosinolate) by the human colonic microflora in a dynamic in vitro large-intestinal model. Carcinogenesis 23:1009–1016
66. Kushad MM, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, Jeffery EH (1999) Variation of glucosinolates in vegetable crops of Brassica oleracea. J Agric Food Chem 47:1541–1548
67. Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, Kensler TW (2003) Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. J Biol Chem 278:8135–8145
68. Kyung KH, Fleming HP, Young CT, Haney CA (1995) 1-Cyano-2,3-epithiopropane as the primary sinigrin hydrolysis product of fresh cabbage. J Food Sci 60:157–159
69. Lambrix V, Reichelt M, Mitchell-Olds T, Kliebenstein DJ, Gershenzon J (2001) The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences Trichoplusia ni herbivory. Plant Cell 13:2793–2807
70. Lee SH, Kim JS, Yamaguchi K, Eling TE, Baek SJ (2005) Indole-3-carbinol and 3,3'-diindolylmethane induce expression of NAG-1 in a p53-independent manner. Biochem Biophys Res Commun 328:63–69
71. Li Y, Li X, Sarkar FH (2003) Gene expression profiles of I3C- and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis. J Nutr 133:1011–1019
72. Link LB, Potter JD (2004) Raw versus cooked vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev 13:1422–1435
73. London SJ, Yuan J-M, Chung F-L, Gao Y-T, Coetzee GA, Ross RK, Yu MC (2000) Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet 356:724–729
74. Lund E (2003) Non-nutritive bioactive constituents of plants: dietary sources and health benefits of glucosinolates. Int J Vitam Nutr Res 73:135–143
75. Ma Q, Kinneer K, Bi Y, Chan JY, Kan YW (2004) Induction of murine NAD(P)H:quinone oxidoreductase by 2,3,7,8-tetrachlorodibenzo-p-dioxin requires the CNC (cap 'n' collar) basic leucine zipper transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2): cross-interaction between AhR (aryl hydrocarbon receptor) and Nrf2 signal transduction. Biochem J 377:205–213
76. MacLeod AJ, Rossiter JT (1987) Degradation of 2-hydroxybut-3-enyl-glucosinolate (progoitrin). Phytochemistry 26:669–673
77. Maheo K, Morel F, Langouet S, Kramer H, Le Ferrec E, Ketterer B, Guillouzo A (1997) Inhibition of cytochromes P-450 and induction of glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. Cancer Res 57:3649–3652
78. March TH, Jeffery EH, Wallig MA (1998) The cruciferous nitrile, crambene, induces rat hepatic and pancreatic glutathione S-transferases. Toxicol Sci 42:82–90
79. Matsuzaki Y, Koyama M, Hitomi T, Kawanaka M, Sakai T (2004) Indole-3-carbinol activates the cyclin-dependent kinase inhibitor p15(INK4b) gene. FEBS Lett 576:137–140
80. Matusheski NV, Jeffery EH (2001) Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. J Agric Food Chem 49:5743–5749
81. Matusheski NV, Juvik JA, Jeffery EH (2004) Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. Phytochemistry 65:1273–1281
82. Matusheski NV, Swarup R, Juvik JA, Mithen R, Bennett M, Jeffery EH (2006) Epithiospecifier protein from Broccoli (Brassica oleracea L. ssp. italica) inhibits formation of the anticancer agent sulforaphane. J Agric Food Chem 54:2069–2076
83. McDanell R, McLean AE, Hanley AB, Heaney RK, Fenwick GR (1988) Chemical and biological properties of indole glucosinolates (glucobrassicins): a review. Food Chem Toxicol 26:59–70
84. McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C, Hayes JD (2001) The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. Cancer Res 61:3299–3307
85. McMahon M, Itoh K, Yamamoto M, Hayes JD (2003) Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. J Biol Chem 278:21592–21600
86. McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD (2006) Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. J Biol Chem 281:24756–24768
87. McWalter GK, Higgins LG, McLellan LI, Henderson CJ, Song L, Thornalley PJ, Itoh K, Yamamoto M, Hayes JD (2004) Transcription factor Nrf2 is essential for induction of NAD(P)H:quinone oxidoreductase 1, glutathione S-transferases, and glutamate cysteine ligase by broccoli seeds and isothiocyanates. J Nutr 134:3499S–3506S
88. Miao W, Hu L, Scrivens PJ, Batist G (2005) Transcriptional regulation of NF-E2 p45-related factor (NRF2) expression by the aryl hydrocarbon receptor-xenobiotic response element signaling pathway: direct cross-talk between phase I and II drug-metabolizing enzymes. J Biol Chem 280:20340–20348
89. Mithen R (2001) Glucosinolates and their degradation products. Adv Bot Res 35:214–262
90. Mithen R, Faulkner K, Magrath R, Rose P, Williamson G, Marquez J (2003) Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Genet 106:727–734

91. Morimitsu Y, Nakagawa Y, Hayashi K, Fujii H, Kumagai T, Nakamura Y, Osawa T, Horio F, Itoh K, Iida K, Yamamoto M, Uchida K (2002) A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. J Biol Chem 277:3456–3463
92. Myzak MC, Dashwood WM, Orner GA, Ho E, Dashwood RH (2006) Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apc min mice. FASEB J 20:506–508
93. Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E (2006) Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. Carcinogenesis 27:811–819
94. Myzak MC, Karplus PA, Chung FL, Dashwood RH (2004) A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. Cancer Res 64:5767–5774
95. Nakajima M, Yoshida R, Shimada N, Yamazaki H, Yokoi T (2001) Inhibition and inactivation of human cytochrome P450 isoforms by phenethyl isothiocyanate. Drug Metab Dispos 29:1110–1113
96. Nakamura Y, Morimitsu Y, Uzu T, Ohigashi H, Murakami A, Naito Y, Nakagawa Y, Osawa T, Uchida K (2000) A glutathione S-transferase inducer from papaya: rapid screening, identification and structure-activity relationship of isothiocyanates. Cancer Lett 157:193–200
97. Nakamura Y, Ohigashi H, Masuda S, Murakami A, Morimitsu Y, Kawamoto Y, Osawa T, Imagawa M, Uchida K (2000) Redox regulation of glutathione S-transferase induction by benzyl isothiocyanate: correlation of enzyme induction with the formation of reactive oxygen intermediates. Cancer Res 60:219–225
98. Nebert DW, Dalton TP, Okey AB, Gonzalez FJ (2004) Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J Biol Chem 279:23847–23850
99. Nioi P, Hayes JD (2004) Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. Mutat Res 555:149–171
100. Nioi P, McMahon M, Itoh K, Yamamoto M, Hayes JD (2003) Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. Biochem J 374:337–348
101. Noda S, Harada N, Hida A, Fujii-Kuriyama Y, Motohashi H, Yamamoto M (2003) Gene expression of detoxifying enzymes in AhR and Nrf2 compound null mutant mouse. Biochem Biophys Res Commun 303:105–111
102. Petroski RJ, Tookey HV (1982) Interactions of thioglucoside glucosinolase and epithiospecifier protein of cruciferous plants to form 1-cyano-epithioalkanes. Phytochemistry 21:1903–1905
103. Plummer SM, Holloway KA, Manson MM, Munks RJ, Kaptein A, Farrow S, Howells L (1999) Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. Oncogene 18:6013–6020
104. van Poppel G, Verhoeven DT, Verhagen H, Goldbohm RA (1999) Brassica vegetables and cancer prevention. Epidemiology and mechanisms. Adv Exp Med Biol 472:159–168
105. Preobrazhenskaya MN, Bukhman VM, Korolev AM, Efimov SA (1993) Ascorbigen and other indole-derived compounds from Brassica vegetables and their analogs as anticarcinogenic and immunomodulating agents. Pharmacol Ther 60:301–313
106. Prochaska HJ, Talalay P (1988) Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. Cancer Res 48:4776–4782
107. Rahman KM, Li Y, Sarkar FH (2004) Inactivation of Akt and NF-κB plays important roles during I3C-induced apoptosis in breast cancer cells. Nutr Cancer 48:84–94
108. Rahman KW, Sarkar FH (2005) Inhibition of nuclear translocation of nuclear factor-κB contributes to 3,3′-diindolylmethane-induced apoptosis in breast cancer cells. Cancer Res 65:364–371
109. Ramsdell HS, Eaton DL (1988) Modification of aflatoxin B1 biotransformation in vitro and DNA binding in vivo by dietary broccoli in rats. J Toxicol Environ Health 25:269–284
110. Rosa EA, Heaney RK, Fenwick GR, Portas CA (1997) Glucosinolates in crop plants. Hortic Rev 19:99–215
111. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG (2000) Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature 403:103–108
112. Sanderson JT, Slobbe L, Lansbergen GWA, Safe S, van den Berg M (2001) 2,3,7,8-Tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1 and 19 in H295R adrenocortital carcinoma cells. Toxicol Sci 61:40–48
113. Shen G, Khor TO, Hu R, Yu S, Nair S, Ho CT, Reddy BS, Huang MT, Newmark HL, Kong AN (2007) Chemoprevention of familial adenomatous polyposis by natural dietary compounds sulforaphane and dibenzoylmethane alone and in combination in $Apc^{Min/+}$ mouse. Cancer Res 67:9937–9944
114. Shin S, Wakabayashi N, Misra V, Biswal S, Lee GH, Agoston ES, Yamamoto M, Kensler TW (2007) NRF2 modulates aryl hydrocarbon receptor signaling: influence on adipogenesis. Mol Cell Biol 27:7188–7197
115. Singh SV, Herman-Antosiewicz A, Singh AV, Lew KL, Srivastava SK, Kamath R, Brown KD, Zhang L, Baskaran R (2004) Sulforaphane-induced G2/M phase cell cycle arrest involves checkpoint kinase 2-mediated phosphorylation of cell division cycle 25C. J Biol Chem 279:25813–25822
116. Singh SV, Srivastava SK, Choi S, Lew KL, Antosiewicz J, Xiao D, Zeng Y, Watkins SC, Johnson CS, Trump DL, Lee YJ, Xiao H, Herman-Antosiewicz A (2005) Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. J Biol Chem 280:19911–19924
117. Smith TK, Lund EK, Parker ML, Clarke RG, Johnson IT (2004) Allyl-isothiocyanate causes mitotic block, loss of cell adhesion and disrupted cytoskeletal structure in HT29 cells. Carcinogenesis 25:1409–1415
118. Song L, Morrison JJ, Botting NP, Thornalley PJ (2005) Analysis of glucosinolates, isothiocyanates and amine degradation products in vegetable extracts and blood plasm by LC-MS/MS. Anal Biochem 347:234–243
119. Srivastava SK, Singh SV (2004) Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. Carcinogenesis 25:1701–1709
120. Staub RE, Feng C, Onisko B, Bailey GS, Firestone GL, Bjeldanes LF (2002) Fate of indole-3-carbinol in cultured human breast tumor cells. Chem Res Toxicol 15:101–109

121. Talalay P, Fahey JW, Healy ZR, Wehage SL, Benedict AL, Min C, Dinkova-Kostova AT (2007) Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. Proc Natl Acad Sci USA 104:17500–17505
122. Tang L, Zhang Y (2004) Dietary isothiocyanates inhibit the growth of human bladder carcinoma cells. J Nutr 134:2004–2010
123. Tang L, Zhang Y (2005) Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol Cancer Ther 4:1250–1259
124. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S (2002) Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 62:5196–5203
125. Tijet N, Boutros PC, Moffat ID, Okey AB, Tuomisto J, Pohjanvirta R (2006) Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries. Mol Pharmacol 69:140–153
126. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, Talalay P (2005) Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc Natl Acad Sci USA 101:2040–2045
127. Wang XJ, Hayes JD, Henderson CJ, Wolf CR (2007) Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha. Proc Natl Acad Sci USA 104:19589–19594
128. Wentzell AM, Rowe HC, Hansen BG, Ticconi C, Halkier BA, Kliebenstein DJ (2007) Linking metabolic QTLs with network and cis-eQTLs controlling biosynthetic pathways. PLoS Genet 3:1687–701
129. World Cancer Research Fund/American Institute for Cancer Research (1997) Food, nutrition and the prevention of cancer. AICR, Washington, DC
130. Xiao D, Johnson CS, Trump DL, Singh SV (2004) Proteasome-mediated degradation of cell division cycle 25C and cyclin-dependent kinase 1 in phenethyl isothiocyanate-induced G2-M-phase cell cycle arrest in PC-3 human prostate cancer cells. Mol Cancer Ther 3:567–575
131. Xiao D, Singh SV (2002) Phenethyl isothiocyanate-induced apoptosis in p53-deficient PC-3 human prostate cancer cell line is mediated by extracellular signal-regulated kinases. Cancer Res 62:3615–3619
132. Xiao D, Zeng Y, Choi S, Lew KL, Nelson JB, Singh SV (2005) Caspase-dependent apoptosis induction by phenethyl isothiocyanate, a cruciferous vegetable-derived cancer chemopreventive agent, is mediated by Bak and Bax. Clin Cancer Res 11:2670–2679
133. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, Conney AH, Kong AN (2006) Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res 66:8293–6
134. Xu C, Shen G, Chen C, Gélinas C, Kong AN (2005) Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. Oncogene 24:4486–4495
135. Xu K, Thornalley PJ (2000) Studies on the mechanism of the inhibition of human leukaemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro. Biochem Pharmacol 60:221–231
136. Xu K, Thornalley PJ (2001) Involvement of glutathione metabolism in the cytotoxicity of the phenethyl isothiocyanate and its cysteine conjugate to human leukaemia cells in vitro. Biochem Pharmacol 61:165–177
137. Xu K, Thornalley PJ (2001) Signal transduction activated by the cancer chemopreventive isothiocyanates: cleavage of BID protein, tyrosine phosphorylation and activation of JNK. Br J Cancer 84:670–673
138. Youle RJ, Strasser A (2008) The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 9:47–59
139. Zabala M de T, Grant M, Bones AM, Bennett R, Lim YS, Kissen R, Rossiter JT (2005) Characterisation of recombinant epithiospecifier protein and its over-expression in Arabidopsis thaliana. Phytochemistry 66:859–867
140. Zhang Y (2000) Role of glutathione in the accumulation of anticarcinogenic ITCs and their glutathione conjugates by murine hepatoma cells. Carcinogenesis 21:1175–1182
141. Zhang DD, Hannink M (2003) Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 23:8137–8151
142. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P (1994) Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci USA 91:3147–3150
143. Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M (2004) Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol 24:10941–10953
144. Zhang Z, Ober JA, Kliebenstein DJ (2006) The gene controlling the quantitative trait locus EPITHIOSPECIFIER MODIFIER1 alters glucosinolate hydrolysis and insect resistance in Arabidopsis. Plant Cell 18:1524–36
145. Zhang J, Svehlíková V, Bao Y, Howie AF, Beckett GJ, Williamson G (2003) Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. Carcinogenesis 24:497–503
146. Zhang Y, Talalay P (1998) Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic Phase 2 enzymes. Cancer Res 58:4632–4639
147. Zhang Y, Talalay P, Cho CG, Posner GH (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci USA 89:2399–2403
148. Zhang Y, Tang L, Gonzalez V (2003) Selected isothiocyanates rapidly induce growth inhibition of cancer cells. Mol Cancer Ther 2:1045–1052
149. Zhou W, Lo SC, Liu JH, Hannink M, Lubahn DB (2007) ERRbeta: a potent inhibitor of Nrf2 transcriptional activity. Mol Cell Endocrinol 278:52–62