Cell. Mol. Life Sci. 64 (2007) 1105 – 1127
1420-682X/07/091105-23
DOI 10.1007/s00018-007-6484-5

Cellular and Molecular Life Sciences

Review

Molecular basis for chemoprevention by sulforaphane: a comprehensive review

N. Juge[a,b], R. F. Mithen[a] and M. Traka[a,*]

[a] Phytochemicals and Health Programme, Institute of Food Research, Colney Lane, Norwich, NR4 7UA (UK), Fax: +44 (0)1603 507723, e-mail: maria.traka@bbsrc.ac.uk
[b] Biosciences FRE-3005-CNRS, Université Paul Cézanne Aix Marseille III, Av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 20 (France)

Received: 10 November 2006; received after revision 15 January 2007; accepted 5 February 2007
Online First 31 March 2007

Abstract. The consumption of cruciferous vegetables has long been associated with a reduced risk in the occurrence of cancer at various sites, including the prostate, lung, breast and colon. This protective effect is attributed to isothiocyanates present in these vegetables, and sulforaphane (SF), present in broccoli, is by far the most extensively studied to uncover the mechanisms behind this chemoprotection. The major mechanism by which SF protects cells was traditionally thought to be through Nrf2-mediated induction of phase 2 detoxification enzymes that elevate cell defense against oxidative damage and promote the removal of carcinogens. However, it is becoming clear that there are multiple mechanisms activated in response to SF, including suppression of cytochrome P450 enzymes, induction of apoptotic pathways, suppression of cell cycle progression, inhibition of angiogenesis and anti-inflammatory activity. Moreover, these mechanisms seem to have some degree of interaction to synergistically afford chemoprevention.

Keywords. Sulforaphane, Nrf2, apoptosis, cell cycle, inflammation, angiogenesis, cancer.

Introduction

Crucifers belong to the family Brassicaceae and include *Brassica oleracea* (broccoli, cabbage, cauliflower, brussels sprouts), *B. rapa* (Chinese cabbage and turnips) and several salad crops, such as *Rorippa nasturtium-aquaticum* (watercress) and *Eruca sativa* (rocket). Evidence for the protective effect of crucifers comes from epidemiological studies in the US [1–5], Europe [6], Shanghai [7, 8] and Singapore [9, 10], in which associations are reported between consumption of cruciferous vegetables and reduction in cancer risk at several sites, including the lung [4, 5, 8, 10], breast [1, 7], colon and rectum [3, 9] and prostate [2, 11, 12]. Certain studies have specifically quantified broccoli consumption, as opposed to crucifers in general, and shown that a diet rich in broccoli can reduce cancer risk [2].

The protective effect of cruciferous vegetables is likely to be influenced by GSTM1 genotype. Glutathione transferases (GSTs) are dimeric enzymes that catalyze the conjugation of glutathione (GSH) with both xenobiotics, including isothiocyanates (ITCs), and endogenous compounds, thereby facilitating their metabolism and excretion. The cytosolic GSTs are derived from at least 17 subunits within seven classes: alpha (GSTA1–A5), mu (GSTM1–M5), omega (GSTO1, O2), pi (GSTP1), theta (GSTT1, T2) sigma (GSTS1) and zeta (GSTZ1). Approximately 50% of

* Corresponding author.

the population regardless of race have a deletion of the GSTM1 gene ('GSTM1 nulls'). Several epidemiological studies that have associated broccoli consumption with cancer risk have concluded that GSTM1-positive individuals (i.e. those with either one or two GSTM1 alleles) gain greater cancer protection than GSTM1 nulls [2, 4, 5]. It should also be noted that some studies based in Asia, in which the predominant cruciferous vegetable consumed would be Chinese cabbage, have reported the converse, i.e. that GSTM1 nulls may gain more protection [7, 8, 10]. It has been speculated that this may be due to the contrasting chemical properties of the ITCs found in broccoli and Chinese cabbage [13], but further studies into this apparent gene-diet paradox are required.

In this review, we describe aspects of the biological activity of sulforaphane (SF, *R*-1-isothiocyanato-4-methylsulfinylbutane), an ITC obtained in the diet through the consumption of broccoli (*B. oleracea* var *italica*).

Broccoli as the major source of SF

The glucosinolate molecule comprises two parts: a common glycone moiety and a variable aglycone side chain derived from amino acids. Although over 120 different side chain structures have been described, relatively few occur in dietary crucifers (Fig. 1). The major ones are 3-butenyl and 4-pentenyl glucosinolates, and their hydroxylated forms that are predominantly found in Chinese cabbage and other forms of *B. rapa*, but also occur in some forms of *B. oleracea*, and 3-methylthiopropyl, 3-methylsulfinylpropyl, 2-propenyl and 4-methylsulfinylbutyl that are found in *B. oleracea*, such as cabbages, cauliflowers and broccoli. Watercress (*Rorippa* spp.) is the major source of phenylethyl glucosinolate, and rockets (*Diplotaxis* and *Eruca* spp.) contain 4-methylthiobutyl glucosinolate. While all these glucosinolates produce ITCs, the physicochemical properties of the ITCs depend upon their characteristic side chains. Thus, certain ITCs, such as phenylethyl, 2-propenyl and 4-methylthiobutyl have a hot flavor, while 3-butenyl and 4-pentenyl ITCs are more pungent. In contrast to the majority of ITCs, SF contributes little to flavour. It is also the most hydrophilic of all the dietary ITCs. The side chain structure also affects the GSTM1-mediated rate of conjugation with GSH.

Most cultivars of broccoli accumulate between 2 and 10 μmol g^{-1} of 4-methylsulfinyl glucosinolate in their florets. Higher levels on a dry weight basis may sometimes be found within broccoli seedlings ('sprouts') a few days after germination, although these rapidly decline as the seedlings age. A high-glucosinolate variety of broccoli has, however, been specially bred to accumulate about threefold higher levels of glucosinolates in its florets [14].

When raw florets or sprouts are macerated or eaten, between 60 and 80% of the glucosinolate is converted to the SF-nitrile, as opposed to the ITC, due to the combined effects of myrosinase and a non-catalytic protein cofactor ('ESP-like'). However, mild cooking can preserve myrosinase activity while denaturing ESP, resulting in almost 100% conversion to SF. Further cooking denatures myrosinase, and intact glucosinolates are ingested. However, these can be converted to SF in the colon by microbial thioglucosidase activity.

SF is absorbed, conjugated to GSH and then metabolized through the mercapturic pathway, resulting in the excretion of N-acetyl cysteine (NAC) conjugates in the urine. The metabolism of SF depends upon GSTM1 genotype. Gasper and colleagues showed that after 24 h, while GSTM1 null individuals excreted almost 100% of SF that was ingested, GSTM1-positive individuals excreted only about 60% of ingested SF [13].

Anticarcinogenic activity of SF

SF has been shown to be protective against carcinogen-induced tumorigenesis at a variety of sites in rodents. Extracts of 3-day-old broccoli sprouts were highly effective in reducing the incidence, multiplicity and rate of development of mammary tumors in dimethylbenz(a)anthracene (DMBA)-treated rats [15]. SF alone also prevented DMBA-induced preneoplastic lesions in mouse mammary glands [16] and rat mammary tumors [17] and blocked benzo[*a*]pyrene (BaP)-evoked forestomach tumors in ICR mice [18]. Recently, it was shown to decrease the incidence of atypical hyperplasias in pancreatic ducts of hamsters and the incidence and multiplicity of N-nitrosobis(2-oxopropyl)amine (BOP)-initiated adenocarcinomas [19]. It effectively reduced the formation of colonic aberrant crypt foci in azoxymethane (AOM)-treated rats [20] and suppressed the growth of intestinal polyps in the APC^{min} mouse [21]. Recently, SF was shown to inhibit skin tumorigenesis acting prior to the initiation stage in mice [22] and to retard the growth of PC-3 human prostate cancer xenografts in nude mice [23]. Together with its NAC conjugates, it inhibited the growth of lung carcinomas from benign tumors by reducing cell proliferation and inducing apoptosis in the tobacco carcinogen–treated A/J mice [a mixture of 3 μmol BaP and 3 μmol 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] [24]. Taken together these findings suggest that SF can

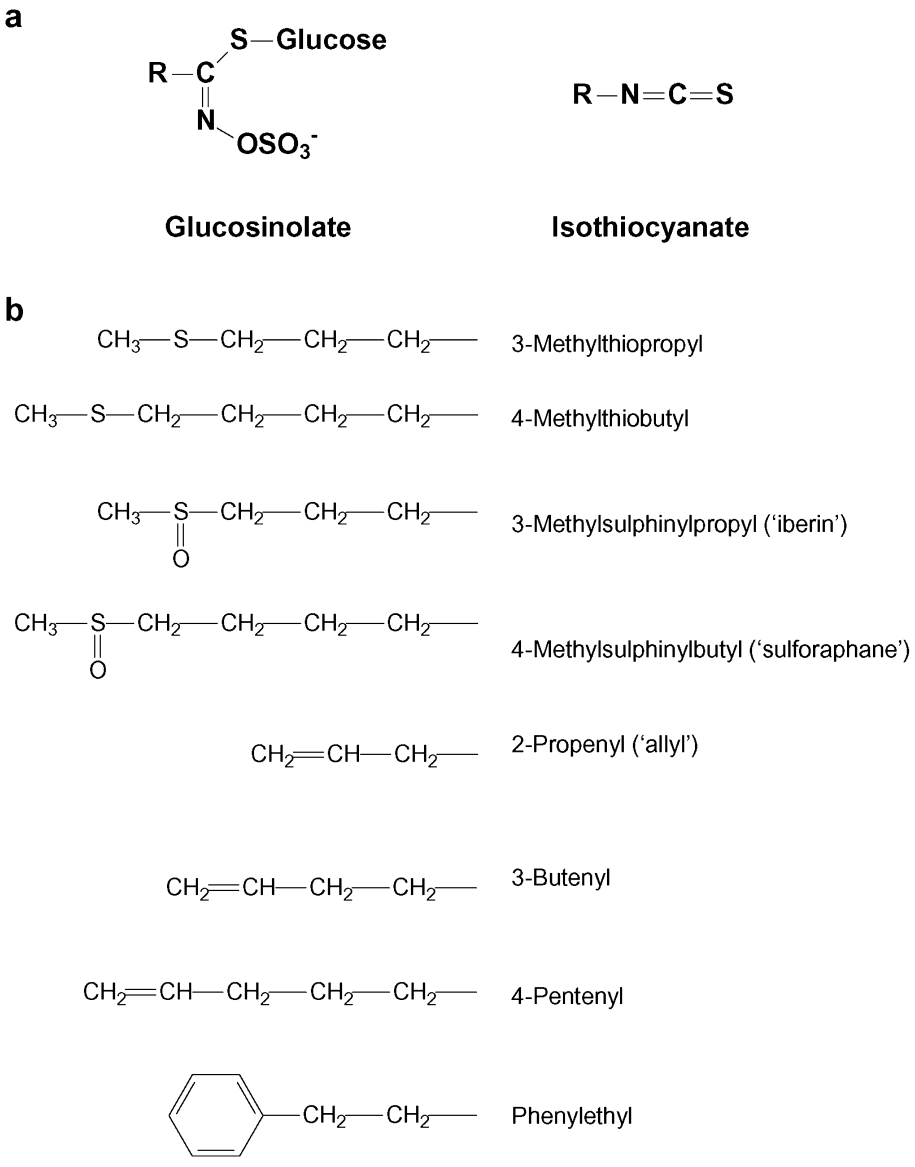

Figure 1. (a) General chemical structure of glucosinolates and isothiocyanates, where R represents the variable side chain. (b) Examples of side chain structures (R) of glucosinolates and isothiocyanates

inhibit the development of adenomas during the initiation and postinitiation stages, thus representing a versatile agent in different stages of early cancer development.

The ultimate chemopreventive effects of SF probably involve multiple mechanisms, which are likely to interact together to reduce the risk of carcinogenesis. These include (a) inhibition of phase 1 cytochrome P450 enzymes, (b) induction of phase 2 metabolism enzymes, (c) antioxidant functions through increased tissue GSH levels, (d) apoptosis-inducing properties, (e) induction of cell cycle arrest, (f) anti-inflammatory properties and (g) inhibition of angiogenesis.

Inhibition of phase 1 enzymes and DNA adducts

Virtually all the dietary and environmental carcinogens are subjected to metabolism once they enter the human body. This enzymatic process occurs mainly through oxidation as well as, to a lesser extent, reduction and hydrolysis, which causes the chemical molecules to become more hydrophilic. This physiological event is called phase 1 metabolism, and is primarily catalyzed by the cytochrome P450 enzymes (CYPs). As a consequence, procarcinogens are usually converted into highly reactive intermediates that can bind to critical macromolecules such as DNA, RNA and protein. So far, 57 CYPs have been identified in humans based on their similarity of DNA sequence and some protein functions [25]. Phase 1 metabolism occurs when ligands bind to the

aryl hydrocarbon (Ah) receptors and the complex is transported to the nucleus to bind to the xenobiotic responsive element (XRE) upstream of cytochrome P450 genes [reviewed in ref. 26]. Phase 1 enzymes typically carry out oxidation and reduction reactions that make carcinogens more water soluble, but at the same time are capable of activating compounds to electrophilic species, which can damage DNA. A large body of data is available, which demonstrates that SF may inhibit DNA-adduct and chemical carcinogenesis through alteration of the level of certain CYP isoforms in rodents via a competitive mechanism as well as by a direct covalent modification [27, 28]. For example, SF decreased enzyme activities in rat hepatocytes associated with CYPs 1A1 and 2B1/2, namely ethoxyresorufin-O-deethylase and pentoxyresorufin-O-dealkylase, in a dose-dependent manner [29]. SF was shown to be a potent competitive inhibitor of CYP2E1 with a K(i) of 37.0 ± 4.5 μM in microsomes from livers of acetone-treated rats and to inhibit the genotoxicity of N-nitrosodimethylamine (NDMA) [30]. Unscheduled DNA synthesis induced by NDMA (33.5 μM) in mouse hepatocytes was inhibited in a dose-dependent manner by SF at 0.064–20 μM [30]. SF (0.1–10 μM) gave a marked inhibition of CYP2E1- and CYP1A2-mediated DNA strand breakage by the carcinogens NDMA and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) [31]. In SF-treated human hepatocytes, although the expression of CYP1A2 was unaffected, the expression of CYP3A4, the major CYP in human liver, was markedly decreased at both mRNA and activity levels [29]. These observations demonstrate that in intact human and rat hepatocytes, SF may cause enzyme inhibition of some but not all CYPs and, in the case of CYP3A4, inhibit both its enzyme activity and its expression, probably mediated by the steroid and xenobiotic receptor [32, 33]. SF, at dietary levels, of intake, led to a decline in CYP2B apoprotein levels and at higher levels decreased CYP3A2 apoprotein levels [34]. In β-naphthoflavone-treated rats, although SF administration upregulated CYP1A2 levels, the enzyme was catalytically inactive, probably due to bound SF metabolite(s) [34].

Associated with the ability of SF to inhibit phase 1 enzymes is its ability to inhibit the formation of carcinogen-induced DNA adducts. *In vitro* antimutagenicity studies strongly suggest that SF is a potent inhibitor of the mutagenicity induced by heterocyclic amines (HCAs) [35]. Exposure to HCAs derived from cooked meat has been implicated in the etiology of certain human cancers including colon, prostate and breast cancer. The total intake of HCAs by an individual can be up to tens of micrograms per day, with 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) being the most abundant type of HCA. Treatment with SF (1–10 μM) significantly reduced the level of PhIP-DNA adducts in human HepG2 cells and human hepatocytes in a dose-dependent manner. The degree of protection was dependent on PhIP concentration, i.e. after 100 pM PhIP exposure, SF reduced adduct levels to below the limit of detection (0.15 amol PhIP/ μg DNA), but at higher PhIP exposure (10 nM and 1 μM), the protection was 60% [36]. SF also increased the rate of detoxification of PhIP in human HepG2 cells but did not affect the rate of PhIP–DNA adduct repair. This protection was not attributed to modulation of CYP1A2 levels, because SF failed to reduce this enzyme, probably due to the low baseline levels in HepG2 cells, but was ascribed to the induction of phase 2 detoxification enzymes (see below).

SF was also shown to inhibit the formation of DNA adducts following BaP and 1,6-dinitropyrene exposure in the human mammary epithelial cell line MCF-10F [37]. Pretreatment of LS-174 cells for 24 h with SF before BaP exposure for 24 h reduced to <20% the number of single-strand DNA breaks produced by the carcinogen and the protection was increased when SF was used in combination with a dietary indole, indolo[3,2-b]carbazole [38]. Non-toxic doses of SF failed to induce expression of CYP1A1 in LS-174 cells, but afforded protection by an increase in the protein levels of phase 2 enzymes [38].

Together these reports provide evidence for protection by SF against carcinogen-DNA damage *in vitro* and suggest a role for inhibition of certain phase 1 enzymes (Fig. 2), although the correlation between the two mechanisms is not always clearly demonstrated.

Induction of phase 2 detoxification enzymes

One important process in chemoprotection by SF involves modulation of the activity of the so-called phase 2 enzymes, which convert carcinogens to inactive metabolites that are readily excreted from the body, thus preventing their reaction with DNA. Although phase 2 enzymes have been traditionally recognized as those catalyzing the conjugation of endogenous ligands, GSH and glucuronic acid, to endo- and xenobiotic substrates, this classification is expanding to include proteins that catalyze a wide variety of reactions that confer cytoprotection against the toxicity of electrophiles and reactive oxygen species (ROS). SF has received much attention over the past decade when it was found to be the most potent naturally occurring inducer of phase 2 enzymes in both animals and humans [39–41], where a strong inverse relationship exists between tissue levels of

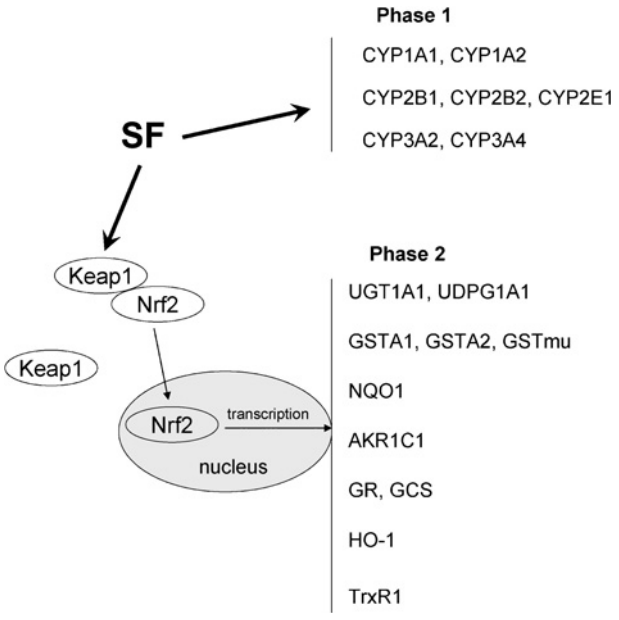

Figure 2. SF modulates the chemoprotective mechanism of cells by altering expression of phase 1 and phase 2 metabolizing enzymes. SF suppresses the phase 1 enzymes responsible for activation of carcinogens and activates phase 2 enzymes through the Nrf2 transcription factor that is responsible for multiple detoxification processes.

these enzymes and susceptibility to chemical carcinogenesis [42].

In vitro, SF is a very potent inducer of quinone reductase (NAD[P]H:quinone oxidoreductase, NQO1, EC 1.6.99.2), glutathione *S*-transferases (GST; EC 2.5.1.18) and UDP-glucuronosyltransferase (UGT; EC 2.4.1.17) amongst others (Fig. 2). The modulation of phase 2 gene expression and enzyme activity by SF has been determined in a number of model cell lines of different origin, the most commonly utilized being derived from liver hepatoma, human HepG2 and mouse Hepa1c1c7, as hepatocellular carcinoma is the most common type of liver cancer and the fourth leading cause of cancer deaths worldwide. For example, SF and its GSH conjugate increased significantly both UGT1A1 and GSTA1 mRNA levels in HepG2 and HT29 cells [43]. When Hepa1c1c7 cells were exposed to increasing levels of SF for 24 h, NQO1 showed a three fold maximal induction over control at 2.5 μM SF [44]. Time- and dose-dependent responses by SF have been reported but the induction level and/or the type of phase 2 enzyme vary with the cell lines as reported in a comparative study testing the effect of 25 μM SF on the enzymatic activities of GST, NQO1, aldo-keto reductase (AKR) and glutathione reductase (GR) in seven well-established mammalian cell lines: HepG2, MCF7, MDA-MB-231, LNCaP, HeLa and HT-29 [45]. Non-toxic doses of SF caused an increase of between 11- and 17-fold in the protein levels of NQO1 but also AKR1C1 and gamma-glutamylcysteine synthetase (GCS), an enzyme that catalyzes the rate-limiting step in GSH synthesis [38].

The effect of SF on phase 2 enzyme modulation has also been studied extensively in prostate cancer, where SF was shown to induce significantly phase 2 enzyme expression and activity in the human prostate cells LNCaP, MDA PCa 2a, MDA PCa 2b, PC-3 and TSU-Pr1 treated with 0.1–15 μM SF [45, 46]. SF produced robust and sustained transcriptional induction of NQO1 gene expression that was accompanied by similar increases in NQO1 enzymatic activity in human prostate cells *in vitro* [46]. More recently, induction of GST and NQO1 was also reported in cultured bladder cells [47].

SF is active not only in carcinogenic cells but also in their normal counterpart and non-transformed cell lines. For example, highly inducible levels of NQO1 protein were detected in the non-transformed rat RL34 epithelial cell line whose phenotypic response to SF more closely recapitulates that observed in rodent liver [48]. SF also induced expression of GST A1/2 isoforms and NQO1 in primary rat hepatocytes in a dose-response and time-course manner [49], although prolonged treatment was required to obtain GST induction levels comparable to those obtained in hepatoma cell lines [29]. Similar results were observed in primary cultures of freshly isolated human hepatocytes where NQO1 expression responded to SF but no significant effects on GSTA1 transcription were seen [50]. SF induced phase 2 detoxification enzymes, UDPG1A1 and GSTA1 mRNA expression in human hepatocytes, although UGT1A1 induction was subject to inter-individual variation [36].

SF, like other ITC compounds effective at inducing phase 2 enzymatic activity in mammalian cells *in vitro*, has been found to be effective at inducing the phase 2 enzyme response *in vivo*. In rats and mice given SF for 4–5 days at high dose levels (up to 1000 μmol/kg per day), increased phase 2 enzyme activities were recorded in the liver, lung, mammary gland, pancreas, stomach, small intestine and colon of the animals [16, 40, 44, 51, 52]. At a low dose (40 μmol/kg per day), SF increased GST and NQO1 activities in the duodenum, forestomach and/or the urinary bladder of the animals, with the greatest effects being seen in the urinary bladder [47, 53]. Similarly, SF produced modest but significant increases in the enzymatic activities of NQO1, total GST and GST-mu in prostate tissue of rats compared to control animals [54]. Finally, SF at dietary doses stimulated in a dose-dependent fashion NQO1 but failed to influence GST, epoxide hydrolase and UGT activities in rats exposed to SF in their drinking water for 10 days, equivalent to daily doses of

3 and 12 mg/kg [34]. In general, GST activity was notably less sensitive than NQO1 to induction by SF. This discrepancy is likely due to the fact that several subclasses of GSTs exist, as described earlier, and strong induction of a particular subunit may be partially masked by background activity of other subunits when total GST activity is measured. Nevertheless, studies in humans also revealed that a high consumption of brassicas led to an increase in GST activity [55, 56]. Thus, it is evident that even at dietary doses, SF can modulate the xenobiotic-metabolizing enzyme systems, shifting the balance of carcinogen metabolism towards detoxification, and this may be an important mechanism of its chemopreventive activity.

The Keap-1-Nrf2 pathway

Induction of phase 2 enzymes by inducers such as SF involves the *cis*-acting antioxidant response element (ARE 5′-(G/A)TGA(G/C)nnnGC(G/A)-3′), a specific DNA-promoter-binding region, which is found in the 5'-flanking region of the phase 2 and antioxidant genes [57]. The transcription of ARE-driven genes is regulated, at least in part, by nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which under normal conditions is sequestered in cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and upon exposure of cells to inducers such as oxidative stress and certain chemopreventive agents dissociates from Keap1, translocates to the nucleus, binds to AREs and transactivates phase 2 detoxifying and antioxidant genes (Fig. 2) [reviewed in ref. 58]. SF is a potent inducer of Nrf2 nuclear accumulation in cells [59]; exposure to SF enables Nrf2 to escape Keap1-dependent degradation, leading to stabilization of Nrf2, increased nuclear localization of Nrf2 and activation of Nrf2-dependent cancer-protective genes [48, 60]. The question of how Keap1 regulates Nrf2 has attracted intense interest, and several models have been suggested. Keap1 has been proposed to tether Nrf2 to cytoplasmic actin filaments, thus preventing its access to the nucleus [61, 62]. More recently, Keap1 was found to modulate nuclear Nrf2 levels by enhancing nuclear export of the transcription factor [63]. Keap1 also regulates the active degradation of Nrf2 [48] by functioning as an adaptor for Cul3-dependent ubiquitination and degradation of Nrf2 [64–67]. Keap1 may also repress Nrf2 activity by transiently shuttling into the nucleus to promote its ubiquitylation [68]. A widely accepted model for Nrf2 nuclear accumulation is that modification of Keap1 cysteines leads directly to dissociation of the Keap1-Nrf2 complex [69, 70]. SF is an electrophile that can react with protein thiols to form thionoacyl adducts and displays a pattern of Keap1 modification distinctly different from ARE inducers that modify Keap1 by alkylation. Moreover, SF treatment *in vivo* does not lead to the accumulation of ubiquitinated Keap1 [71], as has been reported recently *in vitro* [72]. This suggests a novel mechanism for Nrf2 stabilization by Keap1-SF thionoacyl adduct formation [71].

Although the Keap1 anchoring model seems to explain successfully the repression and activation of Nrf2 signaling in response to the changing redox conditions, some controversial observations have been reported recently. It was shown that electrophilic modification of Keap1 alone did not disrupt the Keap1-Nrf2 complex [67]. The Keap1-Nrf2 system has been shown to be perturbed through the knockdown of Keap1 by RNAi [73]. Deletion of the Keap1 gene activates Nrf2 and increases nuclear accumulation of Nrf2 resulting in over-expression of many phase 2 enzymes in mouse hepatocytes [74]. However, with the rapid progress in Nrf2 studies, evidence suggests that Nrf2 may be self-sufficient to sense and transduce oxidative signals into the nucleus, consequently initiating antioxidant gene transcription [75]. Keap1, however, may provide an additional regulation of the quantity of Nrf2 both at basal and at inducible conditions.

The Nrf2–Keap1–ARE signaling pathway itself can be modulated by several upstream kinases including phosphatidylinositol 3-kinase (PI3K), protein kinase C and mitogen-activated protein kinases (MAPKs). SF has recently been found to induce Nrf2-regulated genes in a PI3K-dependent manner [76, 77].

The chemopreventive role of Nrf2 is underscored by the increased susceptibility to carcinogenesis and the loss of chemoprotective efficacy of SF on Nrf2-deficient mice. SF blocked BaP-evoked forestomach tumors in ICR mice and this protection resulted from induction of phase 2 detoxification and antioxidant enzymes, and was abrogated in mice lacking the Nrf2 gene [18]. Nrf2(−/−) mice were also more susceptible to skin tumorigenesis, suggesting that the chemopreventive effects of SF on skin tumorigenesis were mediated and require Nrf2 [22].

ARE elements have been identified in the 5'-flanking regions of many cytoprotective genes including heme oxygenase 1 (HO-1; EC 1.14.99.3), GCS, NQO1 and GST [reviewed in ref. 78]. Several studies have shown that Nrf2 knockout mice exhibited decreased inducible induction of phase 2 detoxification and antioxidant enzymes and were highly sensitive to cytotoxic electrophiles as compared to wild-type mice. For example, whereas SF induced NQO1 and GST enzyme activities in the small intestine of Nrf2(+/+) mice by around 1.5-fold, this induction was essentially abolished in the knockout mice [79]. Similarly, dietary administration of broccoli seeds to Nrf2(+/+) and Nrf2(−/−) mice produced an approximately 1.5-fold

increase in NQO1 and GST activities in the stomach, small intestine and liver of wild-type mice but not in mutant mice; increased transferase activity was associated with elevated levels of GSTA1/2, GSTA3 and GSTM1/2 subunits. These extracts increased significantly the level of GCS in the stomach and the small intestine of Nrf2(+/+) mice but not Nrf2(−/−) mice [80]. The broccoli seed extract increased GSTA3, GSTM1 and NQO1 proteins in mouse embryonic fibroblasts from Nrf2(+/+) but not from Nrf2(−/−) mice [80]. These experiments show that SF is effective at inducing antioxidant and detoxification proteins, both *in vivo* and *ex vivo*, in an Nrf2-dependent manner.

At the molecular level, several Nrf2-dependent genes were found to be SF inducible by comparing transcriptional profiles from the small intestine of Nrf2(+/+) and Nrf2 knockout female mice treated with SF [81]. Analysis of gene expression profiles using the Murine Genome U74Av2 oligonucleotide array identified 26 genes as Nrf2-dependent, SF-inducible genes, and this gene cluster included xenobiotic-metabolizing enzymes such as NQO1, GST and GCS but also GSH biosynthesis enzymes, and NADPH-generating enzymes [81]. Recently, a similar approach was used to identify several clusters of genes dependent on Nrf2 for their expression in the liver of SF-fed wild-type and Nrf2-deficient mice by using Affymetrix oligonucleotide microarrays [82]. The products of genes induced by SF through an Nrf2-dependent pathway were classified as xenobiotic-metabolizing enzymes, antioxidants, ubiquitin/proteasome systems, stress response proteins, kinases and phosphatases, immunity proteins, cell adhesion, cell cycle and cell growth, metabolism, transport proteins and transcription factors. This study also clearly suggested that Nrf2 not only mediated the transcription of phase 2 drug metabolism, but could also regulate the expression of phase 3 transporters [82].

SF and oxidative stress

Oxidative stress – a cellular imbalance between production and elimination of ROS, such as superoxide, hydrogen peroxide and peroxynitrite – is considered to be of major pathophysiological relevance for a variety of pathological processes. Thus, it is valuable to identify compounds, which might act as antioxidants, i.e. compounds that antagonize the deleterious action of ROS on biomolecules. The mode of action of these compounds could be either to scavenge ROS directly or to trigger protective mechanisms inside the cell, thereby resulting in improved defense against ROS.

Although SF is not a direct-acting antioxidant or prooxidant [83], there is substantial evidence that SF acts indirectly to increase the antioxidant capacity of animal cells and their abilities to cope with oxidative stress [84]. Induction of phase 2 enzymes is one means by which SF enhances the cellular antioxidant capacity. Enzymes induced by SF include Nrf2-regulated enzymes such as GSTs and NQO1 which can function as protectors against oxidative stress [85]. SF is also a very potent inducer of HO-1 that catalyzes the conversion of heme to biliverdin which in turn is reduced enzymatically to bilirubin [60, 86, 87]. Among the various genes encoding proteins that possess antioxidant characteristics, HO-1 has attracted particular interest as it is finely upregulated by stress conditions and generates products that might have important biological activities. HO-1 displays antioxidant, antiapoptotic, and anti-inflammatory effects and appears to have a complex role in angiogenesis [reviewed in refs. 88, 89].

To protect themselves from oxidative stress, cells are equipped with reducing buffer systems including the GSH and thioredoxin (Trx) reductase. GSH is an important tripeptide thiol which in addition to being the substrate for GSTs maintains the cellular oxidation–reduction balance and protects cells against free radical species. ITCs accumulate rapidly in cells as a result of conjugation with intracellular thiols, especially GSH, to levels reaching 100- to 200-fold over the extracellular concentrations [90–92]. Exposure of cells to ITCs such as SF leads, at least transiently, to a decrease in the pool of cellular –SH groups, which will undoubtedly render cells more susceptible to oxidative stress and stress-induced damage [93]. Both GSH loss and oxidation have been associated with an increased expression of the rate-limiting enzyme of GSH synthesis, GCS (also known as glutamine-L-cysteine ligase, GLCL) and several other detoxification systems including GSTs and NQO1, in response to the stimulation of the Nrf2–ARE signaling pathway by ITCs [94]. When HepG2 cells were incubated with 5 μM SF for 24 h, significant elevations of GSH content (4.3-fold), NQO1 and GST activity were observed and these elevations were closely related with the total intracellular accumulation [95]. GR also plays a critical role by regenerating reduced GSH from the oxidized form. Surprisingly, GR activities were inhibited consistently by SF in six out of seven cell lines, Hepa1c1c7, HepG2, MCF7, MDA-MB-231, LNCaP and HT-29 cells, but not in HeLa cells, where only a slight induction was observed [45]. SF also induces glutathione peroxidase (Gpx), an enzyme that catalyzes the reduction of organic hydroperoxide and hydrogen peroxide, in human Caco-2 cells [96] and mouse Hepa1c1c7 cells [97].

The other major ubiquitous factor responsible for maintaining proteins in their reduced state is Trx, which is reduced by electrons from NADPH via thioredoxin reductase (TrxR) [reviewed in ref. 98]. Functions of some transcription factors, such as nuclear factor-κB (NF-κB), AP-1 and p53, are mediated by Trx. TrxR can directly remove apoptotic inducers such as H_2O_2 and indirectly activate the antiapoptotic activity of NF-κB and reduced Trx [reviewed in ref. 99]. The predominant cytoplasmic/nuclear form, Trx-1, and the mitochondrial form, Trx-2, both protect against oxidative stress. SF upregulated the expression of the inducible form, TrxR1, in a dose-dependent manner in HepG2 cells [100, 101], Hepa1c1c7 mouse hepatoma cells [97, 102], MCF-7 cells [103], EAhy926 endothelial cells [104] and in LNCaP human cell cultures [unpublished results]. SF accounted for most of the ARE-activated transcriptional induction of these antioxidant genes by broccoli [102]. Together, the changes induced by SF promote cellular defenses against carcinogens by increasing the reductive capacity of the cell. The overall indirect antioxidant role of SF can be attributed to *(*a*)* increased tissue GSH levels, *(*b*)* effects on phase 2 enzyme regulation (antioxidant role of GSTs, NQO1 and HO-1) and *(*c*)* effects on redox enzyme/protein regulation (TrxR, GCS), although the cytoprotection as a result of an initial induction of oxidative stress by SF should not be dismissed, as recently reviewed and discussed by Zhang et al. [83].

Apoptosis mediated by SF

Apoptosis, or programmed cell death, is a highly regulated process that occurs under a range of physiological and pathological conditions as part of the cellular mechanism. Apoptosis plays important roles in the development and maintenance of homeostasis and in the elimination of cells that are damaged or no longer necessary for the organism. Inappropriate regulation of apoptosis may cause serious disorders, such as neural degeneration, autoimmune diseases and cancers.

In contrast to necrosis, during which cells suffer a major insult resulting in loss of membrane integrity and uncontrolled release of cellular contents into the environment of the cell, apoptosis is a highly regulated process [105]. The morphological features of apoptosis include cell shrinkage, chromatin condensation and fragmentation of the cell into compact membrane-enclosed structures, called 'apoptotic bodies' that are engulfed by macrophages and removed from the tissue in a controlled manner. These morphological changes are a consequence of characteristic molecular and biochemical events occurring in an apoptotic cell, most notably the activation of proteolytic enzymes which eventually mediate the cleavage of DNA into oligonucleosomal fragments as well as the cleavage of a multitude of specific protein substrates which usually determine the integrity and shape of the cytoplasm or organelles.

Apoptosis is induced by a variety of stimuli, including genotoxic compounds and various environmental stresses, which activate intracellularly a family of cysteine proteases, termed caspases, responsible for the initiation and the execution of apoptosis. To date there are a total of 11 human caspases identified and they are commonly used to assess whether apoptosis is induced in different experimental systems either as early or late markers of apoptosis depending on which member is studied [106]. Caspases are synthesized as inactive proenzymes, which are activated by cleavage at specific aspartate residues to active enzymes comprising large (p20) and small (p10) units [107]. Caspase-3 together with -6 and -7 belong to the subfamily of 'effector' caspases, which perform downstream execution steps by cleaving multiple cellular substrates, while the remaining enzymes are 'initiator' caspases, which process and activate the downstream 'effector' caspases [106]. Activation of caspases results in cleavage and inactivation of key cellular proteins including the DNA repair enzyme poly(-ADP-ribose) polymerase (PARP).

Apoptosis can also be induced by a caspase-independent pathway via release of the mitochondrial protein apoptosis inducing factor (AIF) into the cytosol or activation of calpains, a family of calcium-activated cytosolic proteases [108].

The first evidence for a cytostatic effect of SF was reported in human colon cancer cells [109], where decreased viability in HT29 and Caco-2 cells was observed as a result of treatment with 15 and 50 μM, respectively. Subsequently, the classical hallmarks of apoptosis such as chromatin condensation, translocation of phosphatidylserine across the plasma membrane and DNA fragmentation were detected in SF-treated cells from colon [110], prostate [23, 111], medulloblastoma [112] and mouse mammary cells [113]. To date several reports mainly using *in vitro* models demonstrate that SF mediates apoptosis by regulating multiple targets in the apoptotic pathway (Fig. 3).

The role of caspases in SF-mediated apoptosis

Several members of the caspase family, responsible for the execution of apoptosis in higher eukaryotes, have previously been implicated in apoptosis induced by ITCs [112, 114, 115]. The first report on the involvement of caspases in SF-mediated apoptosis was by

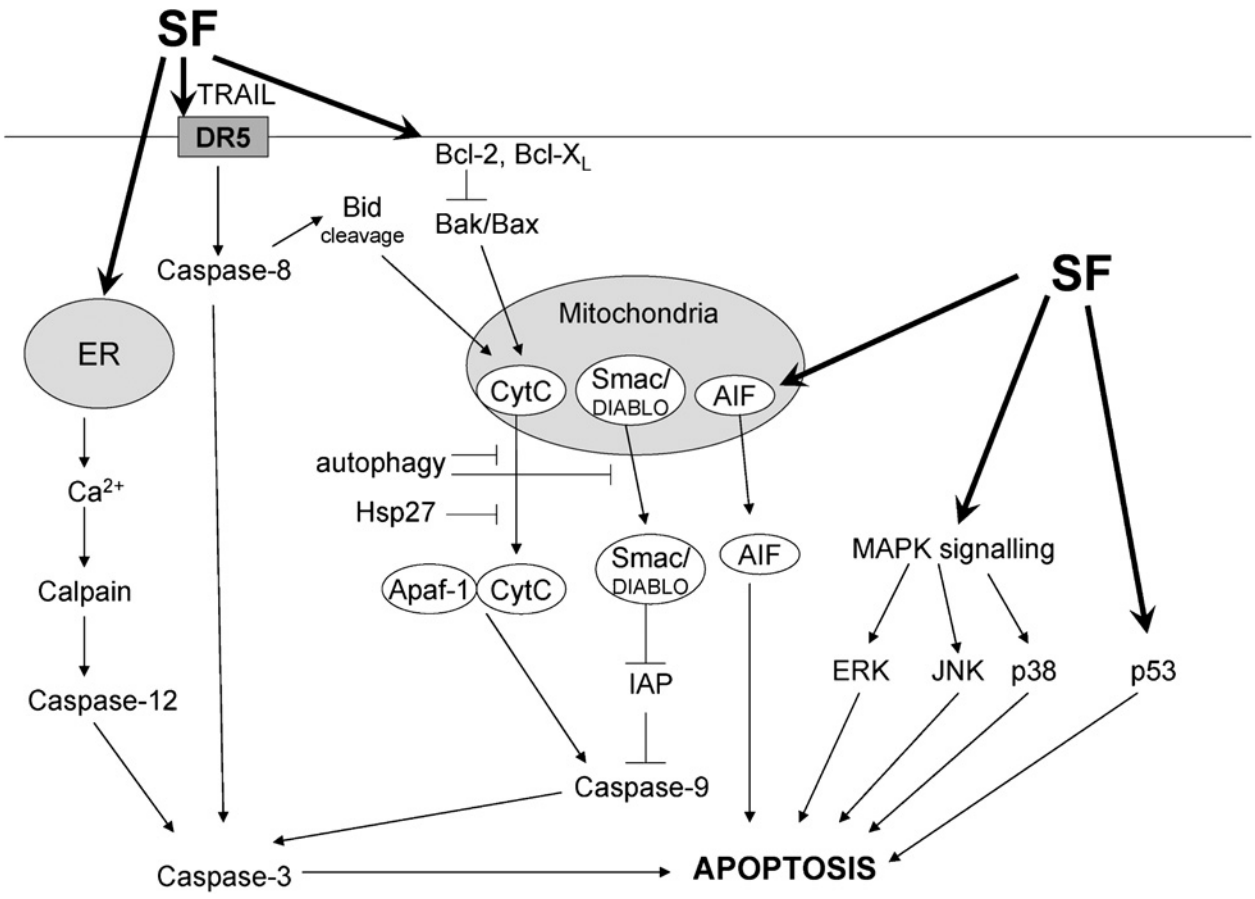

Figure 3. SF induces apoptosis by affecting multiple targets of the mitochondrial/intrinsic and the receptor/extrinsic apoptotic pathways as well as caspase-independent pathways. All the apoptotic components shown in this figure have documented altered expression or (in)activation in response to SF treatment.

Chiao and colleagues in prostate LNCaP cells [116]. Subsequently, SF was shown to activate the mitochondrial/intrinsic apoptotic pathway. This involves release of cytochrome c from the mitochondria into the cytosol, which in turn binds to the apoptosis protease activation factor-1 (Apaf-1) and leads to activation of the 'initiator' caspase-9. Activation of caspase-9 has been described following exposure of human medulloblastoma cells to 10 μM SF [112], 20 μM SF in PC-3 cells [23], 20 μM SF in DU145 cells [117], 10–40 μM SF in mouse embryonic fibroblasts [118] and 20–40 μM SF in human glioblastoma cells [119].

Recently, SF was also shown to activate the death receptor/extrinsic pathway of apoptosis in prostate cancer cells. This pathway is initiated when 'death ligands' such as Fas-L, TRAIL or tumor necrosis factor-α (TNF-α) through their cell surface receptors activate the downstream intracellular apoptotic machinery, which involves induction of caspase-8 and subsequent activation of 'effector' caspases. Treatment of prostate cancerous PC-3 and DU145 cells with SF resulted in activation of caspase-8 and subsequently apoptosis that was abolished in the presence of a specific caspase-8 inhibitor [23, 117]. Similarly, activation of caspase-8 was observed following SF treatment of pancreatic cancer cell lines [120]. The death receptor pathway activated in prostate cells through caspase-8 was responsible for the induction of apoptosis to a greater extent than the mitochondrial pathway through the activation of caspase-9 but both were still active at the same time. Another ITC, phenethyl ITC (PEITC), has been shown to induce both pathways depending on the cell type, by inducing the mitochondrial apoptotic pathway in Hela cells [114] but the death receptor pathway in human leukemia cells [115]. Both pathways, however, converge in the downstream activation of the 'effector' caspase-3, which has been well-documented for SF [23, 112, 118, 119, 121, 122].

Additionally, SF has been shown to activate caspase-12 following damage of the endoplasmic reticulum, implicating this organelle for the first time in SF-mediated apoptosis [119].

The role of mitochondria in SF-mediated apoptosis

Mitochondria play an important role in apoptosis, because permeabilization of their outer membrane and subsequent release of mitochondrial proteins into the cytosol commits the cell to apoptosis. The first protein to be released following permeabilization is cytochrome c that binds to Apaf-1 and subsequently recruits and activates caspase-9. Other mitochondrial proteins include Smac/DIABLO, which blocks members of the inhibitor of apoptosis family (IAP), and the AIF protein, both of which trigger the apoptotic cascade upon release to the cytosol. One way that SF induces apoptosis is by interfering with the mitochondrial integrity. Decrease in mitochondrial potential in response to SF was detected in prostate [123] and bladder cancer cells [124]. Subsequent release of the mitochondrial proteins cytochrome c, Smac/DIABLO and more recently AIF have also been observed [110, 118, 119, 123].

The role of bcl-2 family in SF-mediated apoptosis

Release of proteins, such as cytochrome c and Smac/DIABLO, from the intermembrane space of mitochondria initiates the mitochondrial apoptotic pathway. Members of the Bcl-2 family control this process tightly by blocking the release of any such proteins either through activation of the proapoptotic Bcl-2 members, such as Bax and Bak, or when the anti-apoptotic members, such as Bcl-2 and Bcl-X_L, are activated.

SF-induced apoptosis seems to involve induction of the proapoptotic Bcl-2 family members. Mouse embryonic fibroblasts (MEFs) treated with 40 μM SF showed an increase in the levels of Bax and Bak proteins as early as 4–8 h, which was abolished in SF-treated MEFs derived from Bax/Bak double-knockout mutant mice [118]. Although the levels of the Bcl-2 antiapoptotic protein did not change in SF-treated HT29 cells [110], the Bax:Bcl-2 ratio was significantly increased following SF treatment in PC-3, HepG2 and glioblastoma cells, as a result of an increase in Bax and decrease in Bcl-2 protein levels [23, 119, 122]. The other antiapoptotic protein, Bcl-X_L, did not change in response to SF in PC-3 cells [23] but was significantly decreased in HepG2 cells [122].

Additionally, activated caspase-8 can also engage the intrinsic pathway through cleavage of another Bcl-2 proapoptotic protein, Bid [125]. When PC-3 cells were treated with 40 μM SF, there was an increase in caspase-8 and subsequent cleavage of Bid [123].

The role of ROS in SF-mediated apoptosis

ROS are broadly defined as oxygen-containing chemical species with reactive chemical properties. In biological systems, ROS are constantly generated through a variety of pathways and have destructive actions on both DNA and proteins. To counteract the detrimental effects of ROS, cells have adapted a complex antioxidant enzymatic machinery to maintain redox potential, such as superoxide dismutase, catalase, GSTs and HO-1, as described above.

Induction of ROS can trigger apoptosis in many different systems [126]. More recently, SF-mediated apoptosis was linked with the generation of ROS in prostate cells. Increased levels of ROS were measured as early as 0.5–1 h in DU145, PC-3 and Hep3B cells in response to SF treatment in a dose- and time-dependent manner [117, 123, 127, 128]. Cytosolic release of cytochrome c and apoptosis induction were observed at a later stage in these cells, suggesting that ROS act as upstream molecules in SF-mediated apoptosis.

The role of JNK/MAPK in SF-mediated apoptosis

SF may also induce apoptosis through MAPK signal transduction. MAPKs are important signaling components that convert extracellular signals into intracellular responses through a series of phosphorylation events [129]. There are three distinct but parallel MAPK cascades identified in mammalian cells, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 [130]. Each consists of three kinases, a MAPK kinase kinase (MAPKKK) which phosphorylates and activates a MAPK kinase (MAPKK), which in turn phosphorylates and activates one of the three MAPKs. Although ERKs can be activated by mitogens and growth factors, JNK and p38 can also be activated by many environmental stress stimuli such as UV and γ-irradiation as well as many anticancer drugs, e.g. cisplatinum or etoposide [131, 132].

Activation of the JNK signal transduction pathway has been implicated in ITC-mediated apoptosis death signaling. Activation of all three MAPKs by phosphorylation was observed in HT29 cells treated with PEITC [133] but only ERK and p38 were activated in PC-3 cells [134]. Similarly, SF activated all three MAPKs in HepG2 cells [122], JNK1/2 and ERK1/2 in PC-3 cells [135] but only JNK in DU145 and HepG2-C8 cells [93, 117]. Inhibiting JNK activity with a pharmacological inhibitor abolished the effect of SF on apoptosis in these cells.

The role of p53 in SF-mediated apoptosis

p53 is one of the most important tumor suppressors in the cell that is often mutated in human cancers. Levels of p53 in unstressed cells are very low but increase as a result of various intracellular and extracellular stresses, including ionizing radiation and DNA-damaging agents. One way that p53 acts is by transcriptional regulation of downstream targets involved in the cell

cycle and apoptosis. Induction of apoptosis is mediated by activation of genes involved in the mitochondrial apoptotic pathway, such as Bax and Apaf-1, the death receptor apoptotic pathway, such as KILLER/DR5, or by suppression of cell survival genes, such as Bcl-2 and survivin [136]. The involvement of p53 in SF-mediated apoptosis is not clear. Reports from Fimognari and colleagues suggest that SF treatment increased the expression of p53 protein in non-transformed human T lymphocytes [137], and this increase was associated with a significant increase in the protein levels of Bax and a slight decrease in Bcl-2 measured in T cell leukaemia cells [138]. In contrast, levels of p53 and Bcl-2 remained unchanged in response to SF treatment in HT29 cells [110]. In LNCaP cells, SF was responsible for the stabilization of p53 protein but knocking down expression by specific siRNA suggested that p53 was not involved in SF-mediated apoptosis [111]. Similarly, a mutated p53 status in cell lines of newborn mouse fibroblasts could not prevent SF-induced apoptosis, suggesting that SF acts by a p53-independent pathway [139]. These results render SF an attractive chemotherapeutic agent for tumors with a p53 mutation.

Other effectors of SF-mediated apoptosis

Other mechanisms by which SF induces apoptosis include regulation of the IAP family of proteins, which inhibit caspase activity. Treatment of MEFs with SF for 24 h resulted in a dose-dependent reduction in the protein levels of X-linked IAP (XIAP) that was independent of Bax or Bak proteins [118]. Similarly, the levels of all three members of the IAP family, XIAP, c-IAP1 and c-IAP2, were markedly reduced on treatment of PC-3 and LNCaP cells with SF [111]. c-IAP1 and c-IAP2 were also decreased with SF treatment in human glioblastoma cells [119]. A similar reduction, however, was not evident when human hepatoma cells were treated with SF, but was only observed following cotreatment with the TNF-related apoptosis-inducing ligand (TRAIL) [128]. Simultaneous treatment with SF and TRAIL resulted in enhancement of TRAIL-induced apoptosis in human hepatoma and osteosarcoma cells and this was mediated by upregulation of death receptor 5 (DR5), a receptor for TRAIL, and subsequent caspase cleavage and activation [128, 140]. However, when combined treatment of SF and TRAIL was applied on human lung cells, caspase-3-dependent apoptosis was induced, without associated decreased levels of XIAP [141].

Another apoptotic pathway responsive to SF is induction of endoplasmic reticulum stress. This was shown in glioblastoma cells where treatment with SF resulted in increased levels of intracellular free Ca^{2+} accompanied by an increase in the protein levels of calpain, a Ca^{2+}-dependent cysteine protease, and subsequent activation of caspase-12 in a dose-dependent manner [119].

Despite the activation of a caspase-dependent apoptotic pathway, SF has also been shown to induce apoptosis in a caspase-independent manner. Treatment of glioblastoma cells with 40 μM SF for 24 h caused a significant increase in the cytosolic protein levels of AIF, a mitochondrial protein that becomes an active cell killer following release in the cytosol [119].

SF as an inhibitor of apoptosis

Interestingly, SF has also been shown to inhibit apoptosis, and to date there are two reports describing this. In the first one, SF treatment with concentrations of up to 50 μM in human colon Caco-2 cells for 24 h failed to activate caspase-3 and subsequent DNA fragmentation associated with apoptosis [142]. This was likely due to the upregulation of heat shock protein 27 (Hsp27), which has been shown to inhibit apoptosis and caspase activation through negative regulation of the mitochondrial proteins cytochrome c and Smac/DIABLO, and other means [143, 144]. Additionally, induction of HO-1 and $p21^{waf1/cip1}$ observed in that study in response to SF treatment has also been associated with inhibition of apoptosis [145]. The second report describes the induction of autophagy in prostate cancer cells treated with SF, during which cells are triggered to die in a non-apoptotic manner without caspase activation [146]. SF-induced autophagy resulted in inhibition of cytochrome c release and subsequent apoptosis in these cells.

Cell cycle regulation by SF

The progression of the cell cycle through the four phases, G1, S, G2 and M, is regulated by cyclin-dependent kinase (CDK) molecules and cyclins, which drive the cell from one phase to the next and in turn are regulated by inhibitors. This process offers many potential targets for chemopreventive agents such as SF.

In normal cells, transition from G1 to S phase during the cell cycle requires the activity of two classes of CDKs, CDK4/6 and CDK2. CDKs cause cell cycle progression by phosphorylating and thereby activating the retinoblastoma tumor suppressor protein (Rb), releasing E2F from negative regulators and facilitating transcription of E2F-responsive genes including cyclins E and A [reviewed in refs. 147, 148]. CDK activity is in turn regulated partly by association with positive and negative regulatory

Table 1. Inhibition of cell cycle progression by SF in different cell lines.

G_O/G1 phase	G2/M phase	S phase
HT29 [109, 151]	HT29 [110, 154]	UM-UC-3 [150]
DU-145 [152]	DU-145 [117]	
LnCap [116]	PC-3 [127]	
T24 [157]	MCF-7 [153]	
Non-transformed T lymphocytes [137]	Jurkat T cells [138]	
	Caco-2 [77]	
	MIA Paca-2 [120]	
	UM-UC-3 [150]	
	F3II [113]	

proteins. CDKs exist predominantly in multiple quaternary complexes, each consisting of a cyclin, a CDK, proliferating cell nuclear antigen (PCNA) and cyclin-dependent kinase inhibitors (CDKIs). CDK4/6 associates with D-type cyclins, whereas CDK2 associates with either cyclin E initially or with cyclin A later in the cell cycle [148].

CDK activity can be inhibited by two different families of cyclin-dependent kinase inhibitors, the INK4 and the Cip/Kip family [149]. INK4 members specifically bind to the catalytic subunits of CDK4 and CDK6 and prevent their association with D-type cyclins. The INK4 family includes four proteins, $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$. In contrast to INK4, Cip/Kip proteins have a broader function in the cell cycle by affecting activities of cyclin D-, E- and A-dependent kinases. These include $p21^{waf1/cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ and were identified as potent inhibitors of cyclin E- and A-dependent CDK2 but as possible positive regulators of cyclin D-dependent kinases [149].

There is evidence that one of the mechanisms by which SF exerts its anticarcinogenic effect is by arresting the cell cycle at different stages of its progression (Fig. 4). This arrest has been documented in colon, prostate, breast, bladder and T cells and resulted in both G_o/G1 and G2/M phase block depending on the cell type (Table 1). Additionally, S phase block as a result of SF treatment has been reported in human UM-UC-3 bladder cells [150].

Consistent with inhibition of the cell cycle in response to SF, transcriptome analysis performed on Caco-2 cells revealed that SF induces expression of several cell cycle related genes, including $p21^{waf1/cip1}$ and GADD45β [142]. Additionally, SF enhanced the expression of KLF4, $PC3^{TIS21/BTG2}$, SMAR1 and CKSHS2, all of which have been associated with regulation of the cell cycle, and decreased expression of members of the minichromosome maintenance family (MCM4 and MCM7) associated with DNA synthesis [142].

Cyclin and CDK regulation by SF

Consistent with a block in the G_O/G1 phase of the cell cycle, SF downregulated expression of the cyclin D1 protein in prostate and colon cells [116, 151] and additionally reduced levels of its associated kinase cdk4 in DU-145 prostate cells [152]. A significant reduction in the levels of cyclin D3 and a slight reduction in cyclin D2 and associated CDK4 and cdk6 were also reported in non-transformed T lymphocytes [137]. Arrest at the G_O/G1 phase of the cell cycle in response to SF in prostate cells was associated with reduced phosphorylation of the Rb tumor suppressor protein that activates the transition from G1 to the S phase [152]. SF also induces cell cycle arrest at the G2/M phase by regulating expression of cyclin B1. In human colon and breast cells, 15 μM SF for up to 48 h increased cyclin B1 protein expression [110, 153]. In HT29 cells, this G2/M phase arrest was achieved by maintaining the cdc2 kinase in its active dephosphorylated form, and was associated with phosphorylation/activation of the Rb protein [154]. In contrast, SF treatment of prostate PC-3 and bladder UM-UC-3 cells resulted in reduced expression of cyclin B1 [127, 150]. The G2/M phase arrest in PC-3 cells activated the DNA damage checkpoint pathway through activation of checkpoint kinase 2 (Chk2) and subsequent phosphorylation of cell division cycle 25C [127]. Finally, S phase arrest reported in UM-UC-3 cells in response to SF treatment was also associated with reduced levels of cyclin A [150].

CDK inhibitor regulation by SF

SF also affects the cell cycle through regulation of CDK inhibitors, which are essential for progression through the four phases. Whereas there is no evidence for regulation of the INK4 family of inhibitors, SF has been shown to upregulate members of the Cip/Kip family. Cip/Kip are tumor suppressor proteins that act as cell cycle checkpoints both for G_O/G1 and G2/M phases by binding either directly to PCNA or to cyclin/

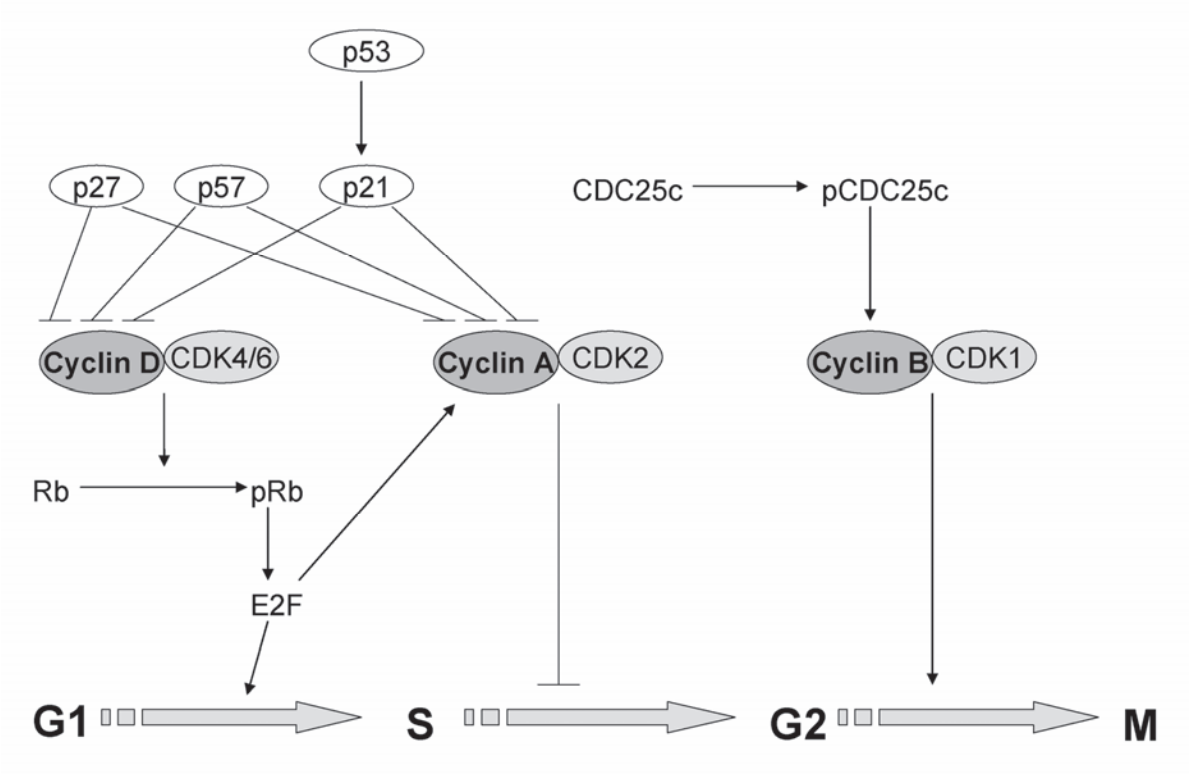

Figure 4. SF arrests the cell cycle by modulating different stages of cell cycle progression. Only members of the cell cycle machinery that have been documented to alter expression or activation status in response to SF treatment are shown.

cdk complexes to block the catalytic activity of the kinase and cause cell cycle arrest [155, 156]. In colon HT29 cells, 15–50 μM SF increased the levels of $p21^{waf1/cip1}$ protein as early as 8 h [151, 154]. Similarly, 9–50 μM SF increased $p21^{waf1/cip1}$ levels in prostate DU-145 cells [152]. Interestingly, in Caco-2 colon cells, SF increased gene expression levels of $p21^{waf1/cip1}$ but decreased $p57^{Kip2}$; the latter when expressed inhibits progression of the cell cycle at the G_O/G1 stage, which is consistent with an SF-induced G2/M phase arrest in these cells [77, 142]. Finally, the only report that SF increases $p27^{Kip1}$ levels comes from treatment of T24 bladder cancer cells [157].

Microtubule disruption by SF

Another possible mechanism for the arrest in cell cycle progression by SF was described by Jackson and colleagues and involved disruption of microtubules by inhibition of tubulin polymerization [113, 153]. A microtubule is a polymer of globular tubulin subunits, consisting of a- and b-tubulins, essential for mitosis, cell motility and transport [158]. The dynamic character of microtubules is expressed through the ability of its subunits to move through the polymer as a result of polymerization at one end and depolymerization at the other. Disruption of tubulin polymerization interferes with mitosis, with a catastrophic outcome for the cell, a process that is targeted by many anticancer drugs, e.g. paclitaxel and taxotere [159]. The first evidence that SF inhibits tubulin polymerization was observed in the mouse mammary carcinoma cell line F3II [113]. In these cells, low concentrations of SF (15 μM) caused mitotic cells to display aberrant and mildly depolymerized spindles, whereas high doses of SF (100–300 μM) inhibited tubulin polymerization. Similar effects were also obtained in bovine endothelial cells [160] and the human breast adenocarcinoma cells MCF-7, where the inhibition of tubulin polymerization was specifically attributed to the ITC group of the SF molecule [153].

Histone modification by SF

Recently, SF was implicated in the modification of histone acetylation, a highly dynamic process that exerts profound control on gene expression by altering chromatin structure. *In vivo*, histone acetylation depends on the balance between the enzymes with histone acetylase activity and enzymes that deacetylate histones (histone deacetylase, HDAC) [161]. Histone acetylation is associated with an open chromatin conformation, allowing for gene transcription, whereas HDACs maintain the chromatin in the closed, non-transcribed state. HDAC is recognized as one of the promising targets for the development of

anticancer drugs as they are usually over-expressed in several tumor cells and tissues [162]. The first report on the effect of SF on histone modification came from human embryonic kidney 293 cells and colon HCT116 cells, where treatment with 15 μM SF decreased HDAC activity and increased acetylated histones H3 and H4 in both cell lines [163]. Interestingly, HDAC inhibition was attributed to the SF metabolites, SF-cysteine and SF-NAC, rather than the SF parent compound. Treatment with 15 μM SF also inhibited HDAC activity in BPH-1, LnCaP and PC-3 prostate cells and increased acetylated levels of H3 and H4 [164]. Additionally, one of the potential mechanisms by which SF increases expression of $p21^{waf1/cip1}$ was found to be increased binding of acetylated H4 to the promoter of $p21^{waf1/cip1}$ [163, 164]. Finally, the results of *in vitro* studies were complemented with *in vivo* work on the APC^{min} mouse model of intestinal carcinogenesis albeit with small group sizes. Six hours post-gavage with 10 μmol SF or its metabolite SF-NAC HDAC activity in the colonic mucosa was reduced, and acetylated H3 and H4 was increased [165]. Ten-week-long feeding with 6 μmol/day SF also resulted in increased acetylated H4 in colonic polyps and to a lesser extent in adjacent normal-looking mucosa [165].

Modulation of inflammation by SF

Although chronic inflammation and carcinogenesis are thought to be mechanistically linked [166], very few data are available regarding anti-inflammatory effects of SF. Chronic inflammation and infections lead to the up-regulation of a series of enzymes and signalling proteins in affected tissues and cells. These proinflammatory enzymes include the inducible forms of nitric oxide (NO) synthase (iNOS) and cyclooxygenase (Cox-2), responsible for elevated levels of NO and prostaglandins (PGs), respectively. Although iNOS provides a benefit to the organism in terms of immune surveillance, aberrant or overproduction of NO has been implicated in the pathogenesis of cancer via reactive NO-species-mediated reactions like nitrosative deamination of DNA bases, lipid peroxidation and DNA strand breaks [167]. Elevated levels in the expression of the inducible Cox-2 have been detected in various tumor types and may account for excessive PG production [168]. In addition to their role as proinflammatory mediators, PGs were demonstrated to suppress immune functions, to inhibit apoptosis, to enhance proliferation and to increase the invasiveness of cancer cells. Consequently, inhibition of expression and enzymatic activity of Cox-2 and downregulation of PG levels is regarded as a rational and feasible strategy in cancer chemoprevention with first positive results in human trials [169]. TNF-α and other inflammatory cytokines were also shown to stimulate tumor promotion and progression of initiated cells as well as of preneoplastic lesions [170] and tumor promotion in TNF-α knockout mice was significantly suppressed in comparison with TNF-α wild-type mice [171]. Thus, TNF-α can be considered as an endogenous tumor promoter and a central mediator in cancer development.

SF down-regulates at the transcriptional level lipopolysaccharide (LPS)-mediated induction of iNOS and Cox-2 expression and TNF-α secretion in cultured raw 264.7 macrophages [172]. NF-κB, a pivotal transcription factor in the LPS-stimulated proinflammatory response, was identified as the key mediator, with SF probably interacting with thiol groups and impairing the redox-sensitive DNA binding and transactivation of NF-κB. SF could either directly inactivate NF-κB subunits by binding to essential cysteine residues or interact with GSH or other redox regulators like Trx and Ref-1 relevant for NF-κB function [172]. SF also significantly decreased NF-κB expression in human malignant glioblastoma compared with corresponding control cells [119].

Additionally, the effects of SF were examined by gene expression profiling in murine BV-2 microglial cells, a neuronal macrophage cell type that mediates inflammatory responses in the brain [173]. In this study, SF was also able to protect cells from H_2O_2-induced toxicity and to attenuate the production of ROS in response to LPS treatment of cells. Similarly, SF attenuated the LPS-induced production and release of proinflammatory mediators [e.g. interleurin (IL)-1β, TNF-α, IL-6] and ROS (e.g. NO) in primary cocultures of rat microglial and astrogial cells [174]. Recently, the effects of SF on the cell-mediated immune response were also investigated in normal as well as tumor-bearing BALB/c mice, where SF significantly enhanced the production of IL-2 and interferon (IFN)-γ and increased the mitogenic potential of various mitogens [175]. Furthermore, recent data suggest that SF may also exert anti-inflammatory effects by inhibiting secretion of high-mobility group box 1 (HMGB1), a nuclear protein that is secreted by immunostimulated macrophages and functions in the extracellular milieu as a proinflammatory mediator. Incubating raw 264.7 cells with SF inhibited both the secretion of LPS-induced HMGB1 and the relocalization of nuclear HMGB1 into the cytoplasm of the cells [176].

The gastrointestinal glutathione peroxidase (GI-GPx, GPx2) has also been implicated in the control of inflammation and cancer, as comprehensively reviewed and discussed by Chu et al. [177] with the

conclusion that an increased expression of GI-GPx prevents cancer by inhibiting preceding inflammation rather than being involved in the development of cancer itself. This enzyme could be another target for SF in limiting inflammation, since it was recently shown that, in Caco-2 cells, GI-GPx was induced by SF via the Nrf2/Keap1 system [96].

Thus, SF may exert anti-inflammatory and anticarcinogenic effects not only by modulation of biotransformation enzymes but also by regulation of genes involved in the inflammation pathway.

Inhibition of angiogenesis and metastasis by SF

Much attention has focused on the ability of SF to slow down the growth of malignancies, such as lung, prostate, breast and colon cancer. While directly targeting the proliferation of mutated cells is important to limiting initial tumor formation, proliferation of the normal population of endothelial cells is a basic requirement for tumor-associated angiogenesis. Indeed, without new blood vessel formation leading to the development of intratumoral capillary networks, tumor progression is limited in growth to only ~0.5 mm in diameter, the maximum tumor thickness through which oxygen and nutrients can diffuse [178]. Hence, investigations aimed toward identifying chemotherapeutic and/or chemopreventive compounds that slow or block the angiogenic response within neoplastic lesions may prove invaluable in limiting later stages of carcinogenesis. While the numerous factors contributing to a tumor's acquisition of an angiogenic phenotype are not well understood, progression of neoplastic angiogenesis requires the presence of certain proangiogenic molecules released from tumor cells [178, 179]. Vascular endothelial growth factor (VEGF) is a central proangiogenic molecule secreted from tumor cells that, aside from favoring endothelial cell survival, acts as a selective endothelial mitogenic, chemotactic and morphogenesis-inducing cytokine [178]. The U.S. Food and Drug Administration recently approved two drugs specifically aimed at VEGF, bevacizumab, a humanized monoclonal antibody, and pegaptinib, a pegylated aptamer with application in ophthalmic pathologies.

Lately, several reports have focused on the effects of SF on endothelial cell functions essential for angiogenesis in several human endothelial cell models. The effects of SF were first investigated on HMEC-1, an immortalized human microvascular endothelial cell line [180]. SF showed time- and concentration-dependent inhibitory effects on hypoxia-induced mRNA expression of VEGF and two angiogenesis-associated transcription factors, hypoxia-inducible factor-1 alpha and c-Myc, in a concentration range of 0.8–25 μM. In addition, the expression of the VEGF receptor KDR/flk-1 was inhibited by SF at the transcriptional level. SF could also affect basement membrane integrity, as it suppressed transcription of the predominant endothelial collagenase matrix metalloproteinase-2 and its tissue inhibitor of metalloproteinase-2. Migration of HMEC-1 cells in a wound-healing assay was effectively prevented by SF at submicromolar concentrations with an IC_{50} of 0.69 μM. In addition, within 6 h of incubation, SF inhibited tube formation of HMEC-1 cells on basement membrane matrix at 0.1, 1 and 10 μM concentrations. These effects were not due to inhibition of HMEC-1 cell proliferation; however, after 72 h of incubation, SF non-selectively reduced HMEC-1 cell growth with an IC_{50} of 11.3 μM [180]. SF also acted to inhibit angiogenesis via suppression of endothelial cell proliferation in bovine aortic endothelial (BAE) cells. Within 24 h, 15 μM SF clearly induced G2/M accumulation and premetaphase arrest in BAE cells [160]. A similar effect was observed in human umbilical vein endothelial cells (HUVECs) where SF induced a dose-dependent decrease in the proliferative activity of cells, which was dependent on cell apoptosis. SF also inhibited tube formation on matrigel, but did not affect matrix metalloproteinase (MMP) production [181]. SF also potently decreased newly formed microcapillaries in a human *in vitro* antiangiogenesis model, with an IC_{50} of 0.08 μM [180]. Furthermore, daily administration of SF (100 nmol/day, intravenously for 7 days) to female Balb/c mice bearing VEGF-impregnated Matrigel plugs strongly and significantly suppressed angiogenesis progression as measured by hemoglobin concentration [160].

Taken together, these findings suggest that the endothelial cell population is a novel target of SF action both *in vitro* and *in vivo*. SF interferes with all essential steps of neovascularization from proangiogenic signaling and basement membrane integrity to endothelial cell proliferation, migration and tube formation. Angiogenesis is a prerequisite for the growth of solid tumors and metastasis. Metastatic cells in the circulation have to perform a series of events to reach a distant site for establishing a new colony. The effect of SF in the inhibition of B16F-10 melanoma-cell-induced metastasis has been studied in C57BL/6 mice [182]. B16F-10 melanoma cells are highly metastatic and form colonies of tumor nodules in the lungs when administered through the tail vein, promoting lung fibrosis and collagen deposition. Metastasis is a multistep process, which involves a series of steps: adhesion of the cancer cells to the basement

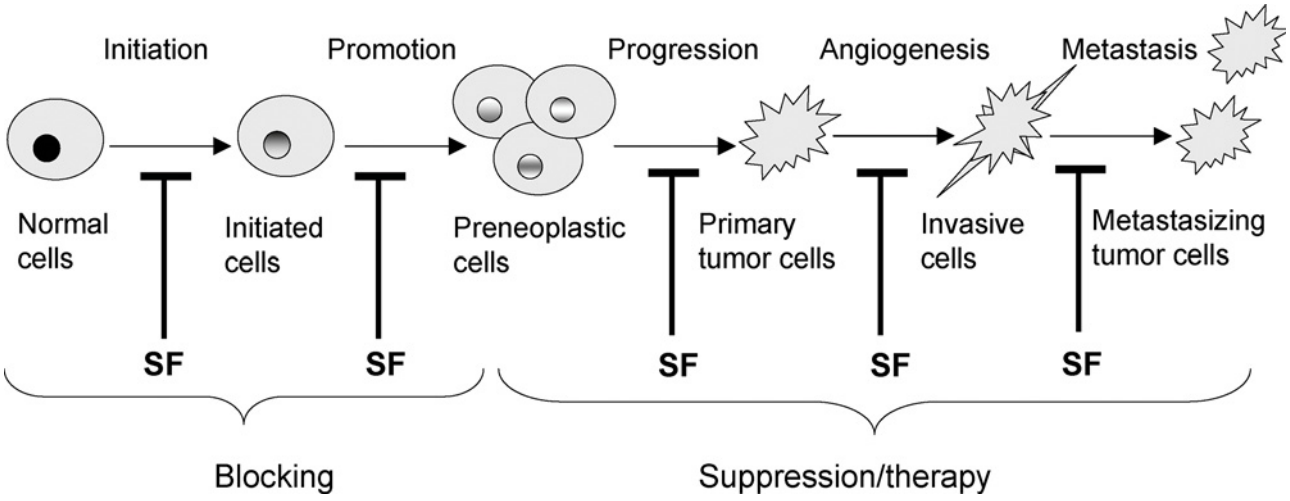

Figure 5. SF acts on the process of carcinogenesis by affecting the three phases tumor initiation, promotion and progression phases, and suppressing the final steps of carcinogenesis, i.e. angiogenesis and metastasis.

membrane, invasion through the basement membrane, circulation, extravasation and proliferation at a new distant site. These findings suggest that SF reduced the invasion of B16F-10 melanoma cells by the inhibition of activation of MMPs, thereby inhibiting lung metastasis [182].

Cross-talk between various metabolic pathways

It is clear that SF can interfere with multiple cell-signaling pathways and has multiple targets within the cell. In addition, some SF molecular targets are themselves involved in overlapping pathways, highlighting the intricate complexity of the overall effect exerted by SF. Examples of this cross-talk between pathways include Nrf2-inducing phase 2 enzymes which are also involved in apoptosis induction [183, 184], MAPK signaling as a starting point for sensing extracellular stresses and inducing Nrf2-mediated transcription or transmitting apoptotic and cell cycle arrest signals [93, 117, 122, 135] and cell cycle checkpoint proteins (e.g. $p21^{waf1/cip1}$) or detoxification enzymes (e.g. HO-1) also inducing apoptosis [145, 185, 186].

SF may also exert antipromotion or antiprogression effects by modulating the NF-κB [59, 121, 187] and activator protein-1 (AP-1) [135, 188] signaling pathways that are usually disregulated in initiated or precancerous cells. Knowledge of cross-talk between NF-κB and Nrf2 is circumstantial but points to HO-1 as an essential link [recently reviewed in ref. 189]. Modulation of NF-κB and AP-1 by SF is far less understood than the Nrf2 signaling pathways. The effect is highly variable depending on the level and duration of the SF treatment and probably the cell types, and diametrically opposite theories exist in current understandings of the relationship between regulation of these signaling pathways and tumorigenesis [reviewed in ref. 190]. These examples highlight the difficulty in predicting phenotypic outcomes from gene expression profiles and the need to look at the overall effect of SF treatment on modulation of both molecular and signal transduction pathways.

Conclusions

In this review, we have presented evidence that SF can interfere with several cell-signaling pathways, including inhibition of carcinogen-activating enzymes, induction of carcinogen-detoxifying enzymes, increase of apoptosis, arrest of cell cycle progression, inhibition of inflammation and angiogenesis, as well as several other mechanisms that are not yet fully described. These mechanisms illustrate the remarkable ability of SF to act on the process of carcinogenesis by affecting the three phases: tumor initiation, promotion and progression phases, and suppressing the final steps of carcinogenesis, i.e. angiogenesis and metastasis (Fig. 5). At an early stage, SF can modulate the enzymes that are required for the activation or detoxification of many carcinogens. In particular, SF has been shown to induce the activity of phase 2 enzymes (e.g. GSTs, NQO1 and glucuronosyltransferases) and/or inhibit phase 1 enzymes (cytochrome P450s) (Fig. 2). In addition, there is now more recent evidence indicating that SF acts to arrest cancerous cell cycle progression, and hence may also retard the development of later stages of carcinogenesis. Possible mechanisms of suppression of tumor development following the initiation of precancerous cells include

the deletion of preinitiated cells from damaged tissues through cell cycle arrest and apoptosis (Fig. 3, 4). Cell cycle arrest by SF occurred through an irreversible G2/M phase arrest via a mechanism involving a reduction of key G2/M–regulating proteins and/or disruption of normal mitotic microtubule polymerization and histone acetylation, whereas overexpression of Bax, downregulation of Bcl-2 and activation of caspase-8 and caspase-9 were implicated in SF-mediated apoptosis. Finally, SF may exert anticarcinogenic effects by the regulation of genes involved in the inflammation pathway and endothelial cell functions, including the proliferation of endothelial cells, tubular formation and MMP production. However, the SF-regulated genes involved in these pathways represent only a subset of those for which expression is modulated by SF or broccoli extracts both in cell cultures and mammalian tissues. There are multiple steps within pathways in which dietary components such as SF can alter gene expression and cell phenotypes and thus influence cancer outcomes (nutritional transcriptomic effect). Analysis of gene expression data have expanded the horizon of SF-regulated genes and molecular pathways, identified novel downstream and upstream mediators for cancer chemoprevention by SF and highlighted the cross-talk between various metabolic pathways. In addition to its effects on protein expression and function, evidence is now accumulating that SF can exert generic effects on the human genome. The next challenge is to determine whether SF in the context of the diet retains its chemopreventive activities through the same molecular mechanisms, outlined in the present review. In particular, questions such as relevant concentrations achieved in the diet, bioavailability, genetic polymorphisms and food matrix interactions should be carefully addressed. Research on nutrient-gene interactions not only provides molecular mechanisms of cancer causation and prevention but also improves the ability to conduct cancer surveillance that is crucial in identifying at-risk populations. Understanding how specific diets or individual dietary compounds alter the balance in favor of chemoprevention will be an important avenue of research. More large-scale clinical trials are also needed to test single nutrients alone or in combination with multiple dietary constituents and functional foods.

1 Ambrosone C. B., McCann, S. E., Freudenheim, J. L., Marshall, J. R., Zhang, Y. and Shields, P. G. (2004) Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. J. Nutr. 134, 1134 – 1138.

2 Joseph, M. A., Moysich, K. B., Freudenheim, J. L., Shields, P. G., Bowman, E. D., Zhang, Y., Marshall, J. R. and Ambrosone, C. B. (2004) Cruciferous vegetables, genetic polymorphisms in glutathione S-transferases M1 and T1, and prostate cancer risk. Nutr. Cancer 50, 206 – 213.

3 Lin, H. J., Probst-Hensch, N. M., Louie, A. D., Kau, I. H., Witte, J. S., Ingles, S. A., Frankl, H. D., Lee, E. R. and Haile, R. W. (1998) Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiol. Biomarkers Prev. 7, 647 – 652.

4 Spitz, M. R., Duphorne, C. M., Detry, M. A., Pillow, P. C., Amos, C. I., Lei, L., de Andrade, M., Gu, X., Hong, W. K. and Wu, X. (2000) Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. Cancer Epidemiol. Biomarkers Prev. 9, 1017 – 1020.

5 Wang, L. I., Giovannucci, E. L., Hunter, D., Neuberg, D., Su, L. and Christiani, D. C. (2004) Dietary intake of cruciferous vegetables, glutathione S-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. Cancer Causes Control 15, 977 – 985.

6 Brennan, P., Hsu, C. C., Moullan, N., Szeszenia-Dabrowska, N., Lissowska, J., Zaridze, D., Rudnai, P., Fabianova, E., Mates, D., Bencko, V., Foretova, L., Janout, V., Gemignani, F., Chabrier, A., Hall, J., Hung, R. J., Boffetta, P. and Canzian, F. (2005) Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach. Lancet 366, 1558 – 1560.

7 Fowke, J. H., Chung, F. L., Jin, F., Qi, D., Cai, Q., Conaway, C., Cheng, J. R., Shu, X. O., Gao, Y. T. and Zheng, W. (2003) Urinary isothiocyanate levels, brassica, and human breast cancer. Cancer Res. 63, 3980 – 3986.

8 London, S. J., Yuan, J. M., Chung, F. L., Gao, Y. T., Coetzee, G. A., Ross, R. K. and Yu, M. C. (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.

9 Seow, A., Yuan, J. M., Sun, C. L., Van Den Berg, D., Lee, H. P. and Yu, M. C. (2002) Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. Carcinogenesis 23, 2055 – 2061.

10 Zhao, B., Seow, A., Lee, E. J., Poh, W. T., Teh, M., Eng, P., Wang, Y. T., Tan, W. C., Yu, M. C. and Lee, H. P. (2001) Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. Cancer Epidemiol. Biomarkers Prev. 10, 1063 – 1067.

11 Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J. and Willett, W. C. (2003) A prospective study of cruciferous vegetables and prostate cancer. Cancer Epidemiol Biomarkers Prev. 12, 1403 – 1409.

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

13 Gasper, A. V., Al-Janobi, A., Smith, J. A., Bacon, J. R., Fortun, P., Atherton, C., Taylor, M. A., Hawkey, C. J., Barrett, D. A. and Mithen, R. F. (2005) Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. Am. J. Clin. Nutr. 82, 1283 – 1291.

14 Mithen, R., Faulkner, K., Magrath, R., Rose, P., Williamson, G. and 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.

15 Fahey, J. W., Zhang, Y. and Talalay, P. (1997) Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. USA 94, 10367 – 10372.

16 Gerhauser, C., You, M., Liu, J., Moriarty, R. M., Hawthorne, M., Mehta, R. G., Moon, R. C. and Pezzuto, J. M. (1997) Cancer chemopreventive potential of sulforamate, a novel analogue of sulforaphane that induces phase 2 drug-metabolizing enzymes. Cancer Res. 57, 272 – 278.

17 Zhang, Y., Kensler, T. W., Cho, C. G., Posner, G. H. and Talalay, P. (1994) Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc. Natl. Acad. Sci. USA 91, 3147 – 3150.
18 Fahey, J. W., Haristoy, X., Dolan, P. M., Kensler, T. W., Scholtus, I., Stephenson, K. K., Talalay, P. and 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.
19 Kuroiwa, Y., Nishikawa, A., Kitamura, Y., Kanki, K., Ishii, Y., Umemura, T. and Hirose, M. (2006) Protective effects of benzyl isothiocyanate and sulforaphane but not resveratrol against initiation of pancreatic carcinogenesis in hamsters. Cancer Lett. 241, 275 – 280.
20 Chung, F. L., Conaway, C. C., Rao, C. V. and Reddy, B. S. (2000) Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. Carcinogenesis 21, 2287 – 2291.
21 Khor, T. O., Hu, R., Shen, G., Jeong, W. S., Hebbar, V., Chen, C., Xu, C., Nair, S., Reddy, B., Chada, K. and Kong, A. N. (2006) Pharmacogenomics of cancer chemopreventive isothiocyanate compound sulforaphane in the intestinal polyps of ApcMin/+ mice. Biopharm. Drug Dispos. 27, 407 – 420.
22 Xu, C., Huang, M. T., Shen, G., Yuan, X., Lin, W., Khor, T. O., Conney, A. H. and Tony Kong, A. N. (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 – 8296.
23 Singh, A. V., Xiao, D., Lew, K. L., Dhir, R. and Singh, S. V. (2004) Sulforaphane induces caspase-mediated apoptosis in cultured PC-3 human prostate cancer cells and retards growth of PC-3 xenografts in vivo. Carcinogenesis 25, 83 – 90.
24 Conaway, C. C., Wang, C. X., Pittman, B., Yang, Y. M., Schwartz, J. E., Tian, D., McIntee, E. J., Hecht, S. S. and Chung, F. L. (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.
25 Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C. and Gotoh, O. (1993) The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol. 12, 1 – 51.
26 Hankinson, O. (1995) The aryl hydrocarbon receptor complex. Annu. Rev. Pharmacol. Toxicol. 35, 307 – 340.
27 Yang, C. S., Smith, T. J. and Hong, J. Y. (1994) Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: opportunities and limitations. Cancer Res. 54, 1982s-1986s.
28 Zhang, Y. and Talalay, P. (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. Cancer Res. 54, 1976s-1981s.
29 Maheo, K., Morel, F., Langouet, S., Kramer, H., Le Ferrec, E., Ketterer, B. and 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.
30 Barcelo, S., Gardiner, J. M., Gescher, A. and Chipman, J. K. (1996) CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane. Carcinogenesis 17, 277 – 282.
31 Barcelo, S., Mace, K., Pfeifer, A. M. and Chipman, J. K. (1998) Production of DNA strand breaks by N-nitrosodimethylamine and 2-amino-3-methylimidazo[4,5-f]quinoline in THLE cells expressing human CYP isoenzymes and inhibition by sulforaphane. Mutat. Res. 402, 111 – 120.
32 Gross-Steinmeyer, K., Stapleton, P. L., Tracy, J. H., Bammler, T. K., Lehman, T., Strom, S. C. and Eaton, D. L. (2005) Influence of Matrigel-overlay on constitutive and inducible expression of nine genes encoding drug-metabolizing enzymes in primary human hepatocytes. Xenobiotica 35, 419 – 438.
33 Zhou, C., Poulton, E. J., Grun, F., Bammler, T. K., Blumberg, B., Thummel, K. E. and Eaton, D. L. (2006) The dietary isothiocyanate, sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor (SXR). Mol. Pharmacol. 71, 220 – 229.
34 Yoxall, V., Kentish, P., Coldham, N., Kuhnert, N., Sauer, M. J. and Ioannides, C. (2005) Modulation of hepatic cytochromes P450 and phase II enzymes by dietary doses of sulforaphane in rats: implications for its chemopreventive activity. Int. J. Cancer 117, 356 – 362.
35 Shishu and Kaur, I. P. (2003) Inhibition of mutagenicity of food-derived heterocyclic amines by sulforaphane – a constituent of broccoli. Indian, J. Exp. Biol. 41, 216 – 219.
36 Bacon, J. R., Williamson, G., Garner, R. C., Lappin, G., Langouet, S. and Bao, Y. (2003) Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. Carcinogenesis 24, 1903 – 1911.
37 Singletary, K. and MacDonald, C. (2000) Inhibition of benzo[a]pyrene- and 1,6-dinitropyrene-DNA adduct formation in human mammary epithelial cells by dibenzoylmethane and sulforaphane. Cancer Lett. 155, 47 – 54.
38 Bonnesen, C., Eggleston, I. M. and Hayes, J. D. (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.
39 Talalay, P. (2000) Chemoprotection against cancer by induction of phase 2 enzymes. Biofactors 12, 5 – 11.
40 Zhang, Y., Talalay, P., Cho, C. G. and Posner, G. H. (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc. Natl. Acad. Sci. USA 89, 2399 – 2403.
41 Prochaska, H. J., Santamaria, A. B. and Talalay, P. (1992) Rapid detection of inducers of enzymes that protect against carcinogens. Proc. Natl. Acad. Sci. USA 89, 2394 – 2398.
42 Kwak, M. K., Egner, P. A., Dolan, P. M., Ramos-Gomez, M., Groopman, J. D., Itoh, K., Yamamoto, M. and Kensler, T. W. (2001) Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. Mutat. Res. 480 – 481, 305 – 315.
43 Basten, G. P., Bao, Y. and Williamson, G. (2002) Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. Carcinogenesis 23, 1399 – 1404.
44 Matusheski, N. V. and Jeffery, E. H. (2001) Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. J. Agric. Food Chem. 49, 5743 – 5749.
45 Jiang, Z. Q., Chen, C., Yang, B., Hebbar, V. and Kong, A. N. (2003) Differential responses from seven mammalian cell lines to the treatments of detoxifying enzyme inducers. Life Sci. 72, 2243 – 2253.
46 Brooks, J. D., Paton, V. G. and Vidanes, G. (2001) Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol. Biomarkers Prev. 10, 949 – 954.
47 Zhang, Y., Munday, R., Jobson, H. E., Munday, C. M., Lister, C., Wilson, P., Fahey, J. W. and Mhawech-Fauceglia, P. (2006) Induction of GST and NQO1 in cultured bladder cells and in the urinary bladders of rats by an extract of broccoli (*Brassica oleracea italica*) sprouts. J. Agric. Food Chem. 54, 9370 – 9376.
48 McMahon, M., Itoh, K., Yamamoto, M. and Hayes, J. D. (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.
49 Payen, L., Courtois, A., Loewert, M., Guillouzo, A. and Fardel, O. (2001) Reactive oxygen species-related induction of multidrug resistance-associated protein 2 expression in

primary hepatocytes exposed to sulforaphane. Biochem. Biophys. Res. Commun. 282, 257 – 263.
50 Gross-Steinmeyer, K., Stapleton, P. L., Liu, F., Tracy, J. H., Bammler, T. K., Quigley, S. D., Farin, F. M., Buhler, D. R., Safe, S. H., Strom, S. C. and Eaton, D. L. (2004) Phytochemical-induced changes in gene expression of carcinogen-metabolizing enzymes in cultured human primary hepatocytes. Xenobiotica 34, 619 – 632.
51 Posner, G. H., Cho, C. G., Green, J. V., Zhang, Y. and Talalay, P. (1994) Design and synthesis of bifunctional isothiocyanate analogs of sulforaphane: correlation between structure and potency as inducers of anticarcinogenic detoxication enzymes. J. Med. Chem. 37, 170 – 176.
52 Keck, A. S., Staack, R. and Jeffery, E. H. (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.
53 Munday, R. and Munday, C. M. (2004) Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds. J. Agric. Food Chem. 52, 1867 – 1871.
54 Jones, S. B. and Brooks, J. D. (2006) Modest induction of phase 2 enzyme activity in the F-344 rat prostate. BMC Cancer 6, 62.
55 Bogaards, J. J., Verhagen, H., Willems, M. I., van Poppel, G. and van Bladeren, P. J. (1994) Consumption of brussels sprouts results in elevated alpha-class glutathione S-transferase levels in human blood plasma. Carcinogenesis 15, 1073 – 1075.
56 Petri, N., Tannergren, C., Holst, B., Mellon, F. A., Bao, Y., Plumb, G. W., Bacon, J., O'Leary, K. A., Kroon, P. A., Knutson, L., Forsell, P., Eriksson, T., Lennernas, H. and Williamson, G. (2003) Absorption/metabolism of sulforaphane and quercetin, and regulation of phase II enzymes, in human jejunum in vivo. Drug Metab. Dispos. 31, 805 – 813.
57 Rushmore, T. H., Morton, M. R. and Pickett, C. B. (1991) The antioxidant responsive element: activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J. Biol. Chem. 266, 11632 – 11639.
58 Jeong, W. S., Jun, M. and Kong, A. N. (2006) Nrf2: a potential molecular target for cancer chemoprevention by natural compounds. Antioxid. Redox Signal 8, 99 – 106.
59 Jakubikova, J., Sedlak, J., Bod'o, J. and Bao, Y. (2006) Effect of isothiocyanates on nuclear accumulation of NF-kappaB, Nrf2, and thioredoxin in caco-2 cells. J. Agric. Food Chem. 54, 1656 – 1662.
60 Jeong, W. S., Keum, Y. S., Chen, C., Jain, M. R., Shen, G., Kim, J. H., Li, W. and Kong, A. N. (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.
61 Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D. and Yamamoto, M. (1999) Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 13, 76 – 86.
62 Kang, M. I., Kobayashi, A., Wakabayashi, N., Kim, S. G. and Yamamoto, M. (2004) Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. Proc. Natl. Acad. Sci. USA 101, 2046 – 2051.
63 Velichkova, M. and Hasson, T. (2005) Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a Crm1-dependent nuclear export mechanism. Mol. Cell Biol. 25, 4501 – 4513.
64 Zhang, D. D., Lo, S. C., Cross, J. V., Templeton, D. J. and Hannink, M. (2004) Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol. Cell Biol. 24, 10941 – 10953.
65 Cullinan, S. B., Gordan, J. D., Jin, J., Harper, J. W. and Diehl, J. A. (2004) The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol. Cell Biol. 24, 8477 – 8486.
66 Furukawa, M. and Xiong, Y. (2005) BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. Mol. Cell Biol. 25, 162 – 171.
67 Eggler, A. L., Liu, G., Pezzuto, J. M., van Breemen, R. B. and Mesecar, A. D. (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.
68 Nguyen, T., Sherratt, P. J., Nioi, P., Yang, C. S. and Pickett, C. B. (2005) Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. J. Biol. Chem. 280, 32485 – 32492.
69 Dinkova-Kostova, A. T., Holtzclaw, W. D., Cole, R. N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M. and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc. Natl. Acad. Sci. USA 99, 11908 – 11913.
70 Zhang, D. D. and 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.
71 Hong, F., Freeman, M. L. and Liebler, D. C. (2005) Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. Chem. Res. Toxicol. 18, 1917 – 1926.
72 Zhang, D. D., Lo, S. C., Sun, Z., Habib, G. M., Lieberman, M. W. and Hannink, M. (2005) Ubiquitination of Keap1, a BTB-Kelch substrate adaptor protein for Cul3, targets Keap1 for degradation by a proteasome-independent pathway. J. Biol. Chem. 280, 30091 – 30099.
73 Devling, T. W., Lindsay, C. D., McLellan, L. I., McMahon, M. and Hayes, J. D. (2005) Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. Proc. Natl. Acad. Sci. USA 102, 7280 – 7285A.
74 Okawa, H., Motohashi, H., Kobayashi, A., Aburatani, H., Kensler, T. W. and Yamamoto, M. (2006) Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochem. Biophys. Res. Commun. 339, 79 – 88.
75 Li, W., Yu, S. W. and Kong, A. N. (2006) Nrf2 possesses a redox-sensitive nuclear exporting signal in the Neh5 transactivation domain. J. Biol. Chem. 281, 27251 – 27263.
76 Svehlikova, V., Wang, S., Jakubikova, J., Williamson, G., Mithen, R. and Bao, Y. (2004) Interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1 in CaCo-2 cells. Carcinogenesis 25, 1629 – 1637.
77 Jakubikova, J., Sedlak, J., Mithen, R. and Bao, Y. (2005) Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G2/M arrest and cell death in Caco-2 cells. Biochem. Pharmacol. 69, 1543 – 1552.
78 Lee, J. S. and Surh, Y. J. (2005) Nrf2 as a novel molecular target for chemoprevention. Cancer Lett. 224, 171 – 184.
79 McMahon, M., Itoh, K., Yamamoto, M., Chanas, S. A., Henderson, C. J., McLellan, L. I., Wolf, C. R., Cavin, C. and Hayes, J. D. (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.
80 McWalter, G. K., Higgins, L. G., McLellan, L. I., Henderson, C. J., Song, L., Thornalley, P. J., Itoh, K., Yamamoto, M. and Hayes, J. D. (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.

81 Thimmulappa, R. K., Mai, K. H., Srisuma, S., Kensler, T. W., Yamamoto, M. and Biswal, S. (2002) Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res. 62, 5196 – 5203.
82 Hu, R., Xu, C., Shen, G., Jain, M. R., Khor, T. O., Gopalkrishnan, A., Lin, W., Reddy, B., Chan, J. Y. and Kong, A. N. (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.
83 Zhang, Y., Li, J. and Tang, L. (2005) Cancer-preventive isothiocyanates: dichotomous modulators of oxidative stress. Free Radic. Biol. Med. 38, 70 – 77.
84 Fahey, J. W. and Talalay, P. (1999) Antioxidant functions of sulforaphane: a potent inducer of phase II detoxication enzymes. Food Chem. Toxicol. 37, 973 – 979.
85 Yanaka, A., Zhang, S., Tauchi, M., Suzuki, H., Shibahara, T., Matsui, H., Nakahara, A., Tanaka, N. and Yamamoto, M. (2005) Role of the nrf-2 gene in protection and repair of gastric mucosa against oxidative stress. Inflammopharmacology 13, 83 – 90.
86 Prestera, T. and Talalay, P. (1995) Electrophile and antioxidant regulation of enzymes that detoxify carcinogens. Proc. Natl. Acad. Sci. USA 92, 8965 – 8969.
87 Keum, Y. S., Yu, S., Chang, P. P., Yuan, X., Kim, J. H., Xu, C., Han, J., Agarwal, A. and Kong, A. N. (2006) Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma hepG2 cells. Cancer Res. 66, 8804 – 8813.
88 Prawan, A., Kundu, J. K. and Surh, Y. J. (2005) Molecular basis of heme oxygenase-1 induction: implications for chemoprevention and chemoprotection. Antioxid. Redox Signal 7, 1688 – 1703.
89 Ryter, S. W., Alam, J. and Choi, A. M. (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol. Rev. 86, 583 – 650.
90 Zhang, Y. (2000) Role of glutathione in the accumulation of anticarcinogenic isothiocyanates and their glutathione conjugates by murine hepatoma cells. Carcinogenesis 21, 1175 – 1182.
91 Zhang, Y. (2001) Molecular mechanism of rapid cellular accumulation of anticarcinogenic isothiocyanates. Carcinogenesis 22, 425 – 431.
92 Callaway, E. C., Zhang, Y., Chew, W. and Chow, H. H. (2004) Cellular accumulation of dietary anticarcinogenic isothiocyanates is followed by transporter-mediated export as dithiocarbamates. Cancer Lett. 204, 23 – 31.
93 Kim, B. R., Hu, R., Keum, Y. S., Hebbar, V., Shen, G., Nair, S. S. and Kong, A. N. (2003) Effects of glutathione on antioxidant response element-mediated gene expression and apoptosis elicited by sulforaphane. Cancer Res. 63, 7520 – 7525.
94 Scharf, G., Prustomersky, S., Knasmuller, S., Schulte-Hermann, R. and Huber, W. W. (2003) Enhancement of glutathione and γ-glutamylcysteine synthetase, the rate limiting enzyme of glutathione synthesis, by chemoprotective plant-derived food and beverage components in the human hepatoma cell line HepG2. Nutr. Cancer 45, 74 – 83.
95 Ye, L. and Zhang, Y. (2001) Total intracellular accumulation levels of dietary isothiocyanates determine their activity in elevation of cellular glutathione and induction of phase 2 detoxification enzymes. Carcinogenesis 22, 1987 – 1992.
96 Banning, A., Deubel, S., Kluth, D., Zhou, Z. and Brigelius-Flohe, R. (2005) The GI-GPx gene is a target for Nrf2. Mol. Cell Biol. 25, 4914 – 4923.
97 Keck, A. S. and Finley, J. W. (2006) Aqueous extracts of selenium-fertilized broccoli increase selenoprotein activity and inhibit DNA single-strand breaks, but decrease the activity of quinone reductase in Hepa 1c1c7 cells. Food Chem. Toxicol. 44, 695 – 703.
98 Nakamura, H. (2005) Thioredoxin and its related molecules: update 2005. Antioxid. Redox Signal 7, 823 – 828.
99 Burke-Gaffney, A., Callister, M. E. and Nakamura, H. (2005) Thioredoxin: friend or foe in human disease? Trends Pharmacol Sci. 26, 398 – 404.
100 Zhang, J., Svehlikova, V., Bao, Y., Howie, A. F., Beckett, G. J. and 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.
101 Hintze, K. J., Wald, K. A., Zeng, H., Jeffery, E. H. and Finley, J. W. (2003) Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. J. Nutr. 133, 2721 – 2727.
102 Hintze, K. J., Wald, K. and Finley, J. W. (2005) Phytochemicals in broccoli transcriptionally induce thioredoxin reductase. J. Agric. Food Chem. 53, 5535 – 5540.
103 Wang, W., Wang, S., Howie, A. F., Beckett, G. J., Mithen, R. and Bao, Y. (2005) Sulforaphane, erucin, and iberin up-regulate thioredoxin reductase 1 expression in human MCF-7 cells. J. Agric. Food Chem. 53, 1417 – 1421.
104 Campbell, L., Howie, F., Arthur, J. R., Nicol, F. and Beckett, G. (2007) Selenium and sulforaphane modify the expression of selenoenzymes in the human endothelial cell line EAhy926 and protect cells from oxidative damage. Nutrition 23, 138 – 144.
105 Kerr, J. F., Wyllie, A. H. and Currie, A. R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239 – 257.
106 Degterev, A., Boyce, M. and Yuan, J. (2003) A decade of caspases. Oncogene 22, 8543 – 8567.
107 Keum, Y. S., Jeong, W. S. and Tony Kong, A. N. (2004) Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutat. Res. 555, 191 – 202.
108 Broker, L. E., Kruyt, F. A. and Giaccone, G. (2005) Cell death independent of caspases: a review. Clin. Cancer Res. 11, 3155 – 3162.
109 Gamet-Payrastre, L., Lumeau, S., Gasc, N., Cassar, G., Rollin, P. and Tulliez, J. (1998) Selective cytostatic and cytotoxic effects of glucosinolates hydrolysis products on human colon cancer cells in vitro. Anticancer Drugs 9, 141 – 148.
110 Gamet-Payrastre, L., Li, P., Lumeau, S., Cassar, G., Dupont, M. A., Chevolleau, S., Gasc, N., Tulliez, J. and Terce, F. (2000) Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer Res. 60, 1426 – 1433.
111 Choi, S., Lew, K. L., Xiao, H., Herman-Antosiewicz, A., Xiao, D., Brown, C. K. and Singh, S. V. (2006) D,L-sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. Carcinogenesis 28, 151 – 162.
112 Gingras, D., Gendron, M., Boivin, D., Moghrabi, A., Theoret, Y. and Beliveau, R. (2004) Induction of medulloblastoma cell apoptosis by sulforaphane, a dietary anticarcinogen from *Brassica* vegetables. Cancer Lett. 203, 35 – 43.
113 Jackson, S. J. and Singletary, K. W. (2004) Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. Carcinogenesis 25, 219 – 227.
114 Yu, R., Mandlekar, S., Harvey, K. J., Ucker, D. S. and Kong, A. N. (1998) Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. Cancer Res. 58, 402 – 408.
115 Xu, K. and Thornalley, P. J. (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.
116 Chiao, J. W., Chung, F. L., Kancherla, R., Ahmed, T., Mittelman, A. and Conaway, C. C. (2002) Sulforaphane and

its metabolite mediate growth arrest and apoptosis in human prostate cancer cells. Int. J. Oncol 20, 631 – 636.
117 Cho, S. D., Li, G., Hu, H., Jiang, C., Kang, K. S., Lee, Y. S., Kim, S. H. and Lu, J. (2005) Involvement of c-Jun N-terminal kinase in G2/M arrest and caspase-mediated apoptosis induced by sulforaphane in DU145 prostate cancer cells. Nutr. Cancer 52, 213 – 224.
118 Choi, S. and Singh, S. V. (2005) Bax and Bak are required for apoptosis induction by sulforaphane, a cruciferous vegetable-derived cancer chemopreventive agent. Cancer Res. 65, 2035 – 2043.
119 Karmakar, S., Weinberg, M. S., Banik, N. L., Patel, S. J. and Ray, S. K. (2006) Activation of multiple molecular mechanisms for apoptosis in human malignant glioblastoma T98G and U87MG cells treated with sulforaphane. Neuroscience 141, 1265 – 1280.
120 Pham, N. A., Jacobberger, J. W., Schimmer, A. D., Cao, P., Gronda, M. and Hedley, D. W. (2004) The dietary isothiocyanate sulforaphane targets pathways of apoptosis, cell cycle arrest, and oxidative stress in human pancreatic cancer cells and inhibits tumor growth in severe combined immunodeficient mice. Mol. Cancer Ther. 3, 1239 – 1248.
121 Jeong, W. S., Kim, I. W., Hu, R. and Kong, A. N. (2004) Modulatory properties of various natural chemopreventive agents on the activation of NF-kappaB signaling pathway. Pharm. Res. 21, 661 – 670.
122 Yeh, C. T. and Yen, G. C. (2005) Effect of sulforaphane on metallothionein expression and induction of apoptosis in human hepatoma HepG2 cells. Carcinogenesis 26, 2138 – 2148.
123 Singh, S. V., Srivastava, S. K., Choi, S., Lew, K. L., Antosiewicz, J., Xiao, D., Zeng, Y., Watkins, S. C., Johnson, C. S., Trump, D. L., Lee, Y. J., Xiao, H. and 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.
124 Tang, L. and Zhang, Y. (2005) Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol. Cancer Ther. 4, 1250 – 1259.
125 Li, H., Zhu, H., Xu, C. J. and Yuan, J. (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94, 491 – 501.
126 Simon, H. U., Haj-Yehia, A. and Levi-Schaffer, F. (2000) Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5, 415 – 418.
127 Singh, S. V., Herman-Antosiewicz, A., Singh, A. V., Lew, K. L., Srivastava, S. K., Kamath, R., Brown, K. D., Zhang, L. and 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.
128 Kim, H., Kim, E. H., Eom, Y. W., Kim, W. H., Kwon, T. K., Lee, S. J. and Choi, K. S. (2006) Sulforaphane sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant hepatoma cells to TRAIL-induced apoptosis through reactive oxygen species-mediated up-regulation of DR5. Cancer Res. 66, 1740 – 1750.
129 Cobb, M. H. and Goldsmith, E. J. (1995) How MAP kinases are regulated. J. Biol. Chem. 270, 14843 – 14846.
130 Cano, E. and Mahadevan, L. C. (1995) Parallel signal processing among mammalian MAPKs. Trends Biochem. Sci. 20, 117 – 122.
131 Chen, Y. R., Wang, X., Templeton, D., Davis, R. J. and Tan, T. H. (1996) The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation: duration of JNK activation may determine cell death and proliferation. J. Biol. Chem. 271, 31929 – 31936.
132 Wada, T. and Penninger, J. M. (2004) Mitogen-activated protein kinases in apoptosis regulation. Oncogene 23, 2838 – 2849.
133 Hu, R., Kim, B. R., Chen, C., Hebbar, V. and Kong, A. N. (2003) The roles of JNK and apoptotic signaling pathways in PEITC-mediated responses in human HT-29 colon adenocarcinoma cells. Carcinogenesis 24, 1361 – 1367.
134 Xiao, D. and Singh, S. V. (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.
135 Xu, C., Shen, G., Yuan, X., Kim, J. H., Gopalkrishnan, A., Keum, Y. S., Nair, S. and Kong, A. N. (2006) ERK and JNK signaling pathways are involved in the regulation of activator protein 1 and cell death elicited by three isothiocyanates in human prostate cancer PC-3 cells. Carcinogenesis 27, 437 – 445.
136 Sun, Y. (2006) p53 and its downstream proteins as molecular targets of cancer. Mol. Carcinog. 45, 409 – 415.
137 Fimognari, C., Nusse, M., Berti, F., Iori, R., Cantelli-Forti, G. and Hrelia, P. (2002) Cyclin D3 and p53 mediate sulforaphane-induced cell cycle delay and apoptosis in non-transformed human T lymphocytes. Cell. Mol. Life Sci. 59, 2004 – 2012.
138 Fimognari, C., Nusse, M., Cesari, R., Iori, R., Cantelli-Forti, G. and Hrelia, P. (2002) Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. Carcinogenesis 23, 581 – 586.
139 Fimognari, C., Sangiorgi, L., Capponcelli, S., Nusse, M., Fontanesi, S., Berti, F., Soddu, S., Cantelli-Forti, G. and Hrelia, P. (2005) A mutated p53 status did not prevent the induction of apoptosis by sulforaphane, a promising anticancer drug. Invest. New Drugs 23, 195 – 203.
140 Matsui, T. A., Sowa, Y., Yoshida, T., Murata, H., Horinaka, M., Wakada, M., Nakanishi, R., Sakabe, T., Kubo, T. and Sakai, T. (2006) Sulforaphane enhances TRAIL-induced apoptosis through the induction of DR5 expression in human osteosarcoma cells. Carcinogenesis 27, 1768 – 1777.
141 Jin, C. Y., Moon, D. O., Lee, J. D., Heo, M. S., Choi, Y. H., Lee, C. M., Park, Y. M. and Kim, G. Y. (in press) Sulforaphane sensitizes tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-mediated apoptosis through downregulation of ERK and Akt in lung adenocarcinoma A549 cells. Carcinogenesis .
142 Traka, M., Gasper, A. V., Smith, J. A., Hawkey, C. J., Bao, Y. and Mithen, R. F. (2005) Transcriptome analysis of human colon Caco-2 cells exposed to sulforaphane. J. Nutr. 135, 1865 – 1872.
143 Chauhan, D., Li, G., Hideshima, T., Podar, K., Mitsiades, C., Mitsiades, N., Catley, L., Tai, Y. T., Hayashi, T., Shringarpure, R., Burger, R., Munshi, N., Ohtake, Y., Saxena, S. and Anderson, K. C. (2003) Hsp27 inhibits release of mitochondrial protein Smac in multiple myeloma cells and confers dexamethasone resistance. Blood 102, 3379 – 3386.
144 Concannon, C. G., Gorman, A. M. and Samali, A. (2003) On the role of Hsp27 in regulating apoptosis. Apoptosis 8, 61 – 70.
145 Liu, Z. M., Chen, G. G., Ng, E. K., Leung, W. K., Sung, J. J. and Chung, S. C. (2004) Upregulation of heme oxygenase-1 and p21 confers resistance to apoptosis in human gastric cancer cells. Oncogene 23, 503 – 513.
146 Herman-Antosiewicz, A., Johnson, D. E. and Singh, S. V. (2006) Sulforaphane causes autophagy to inhibit release of cytochrome C and apoptosis in human prostate cancer cells. Cancer Res. 66, 5828 – 5835.
147 Sherr, C. J. (1996) Cancer cell cycles. Science 274, 1672 – 1677.
148 Sherr, C. J. (2000) The Pezcoller lecture: cancer cell cycles revisited. Cancer Res. 60, 3689 – 3695.
149 Sherr, C. J. and Roberts, J. M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. 13, 1501 – 1512.
150 Tang, L. and Zhang, Y. (2004) Dietary isothiocyanates inhibit the growth of human bladder carcinoma cells. J. Nutr. 134, 2004 – 2010.
151 Shen, G., Xu, C., Chen, C., Hebbar, V. and Kong, A. N. (2006) p53-independent G1 cell cycle arrest of human colon carci-

noma cells HT-29 by sulforaphane is associated with induction of p21CIP1 and inhibition of expression of cyclin D1. Cancer Chemother. Pharmacol. 57, 317 – 327.
152 Wang, L., Liu, D., Ahmed, T., Chung, F. L., Conaway, C. and Chiao, J. W. (2004) Targeting cell cycle machinery as a molecular mechanism of sulforaphane in prostate cancer prevention. Int. J. Oncol. 24, 187 – 192.
153 Jackson, S. J. and Singletary, K. W. (2004) Sulforaphane inhibits human MCF-7 mammary cancer cell mitotic progression and tubulin polymerization. J. Nutr. 134, 2229 – 2236.
154 Parnaud, G., Li, P., Cassar, G., Rouimi, P., Tulliez, J., Combaret, L. and Gamet-Payrastre, L. (2004) Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. Nutr. Cancer 48, 198 – 206.
155 Coqueret, O. (2003) New roles for p21 and p27 cell-cycle inhibitors: a function for each cell compartment? Trends Cell Biol. 13, 65 – 70.
156 Cazzalini, O., Perucca, P., Riva, F., Stivala, L. A., Bianchi, L., Vannini, V., Ducommun, B. and Prosperi, E. (2003) p21CDKN1A does not interfere with loading of PCNA at DNA replication sites, but inhibits subsequent binding of DNA polymerase delta at the G1/S phase transition. Cell Cycle 2, 596 – 603.
157 Shan, Y., Sun, C., Zhao, X., Wu, K., Cassidy, A. and Bao, Y. (2006) Effect of sulforaphane on cell growth, G0/G1 phase cell progression and apoptosis in human bladder cancer T24 cells. Int. J. Oncol. 29, 883 – 888.
158 Nogales, E. (1999) A structural view of microtubule dynamics. Cell. Mol. Life Sci. 56, 133 – 142.
159 Bhalla, K. N. (2003) Microtubule-targeted anticancer agents and apoptosis. Oncogene 22, 9075 – 9086.
160 Jackson, S. J., Singletary, K. W. and Venema, R. C. (2006) Sulforaphane suppresses angiogenesis and disrupts endothelial mitotic progression and microtubule polymerization. Vascul. Pharmacol. 46, 77 – 84.
161 Struhl, K. (1998) Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 12, 599 – 606.
162 Kim, D. H., Kim, M. and Kwon, H. J. (2003) Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents. J. Biochem. Mol. Biol. 36, 110 – 119.
163 Myzak, M. C., Karplus, P. A., Chung, F. L. and Dashwood, R. H. (2004) A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. Cancer Res. 64, 5767 – 5774.
164 Myzak, M. C., Hardin, K., Wang, R., Dashwood, R. H. and Ho, E. (2006) Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. Carcinogenesis 27, 811 – 819.
165 Myzak, M. C., Dashwood, W. M., Orner, G. A., Ho, E. and Dashwood, R. H. (2006) Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apc-minus mice. FASEB J. 20, 506 – 508.
166 Ohshima, H. and Bartsch, H. (1994) Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat. Res. 305, 253 – 264.
167 Graziewicz, M., Wink, D. A. and Laval, F. (1996) Nitric oxide inhibits DNA ligase activity: potential mechanisms for NO-mediated DNA damage. Carcinogenesis 17, 2501 – 2505.
168 Subbaramaiah, K., Zakim, D., Weksler, B. B. and Dannenberg, A. J. (1997) Inhibition of cyclooxygenase: a novel approach to cancer prevention. Proc. Soc. Exp. Biol. Med. 216, 201 – 210.
169 Steinbach, G., Lynch, P. M., Phillips, R. K., Wallace, M. H., Hawk, E., Gordon, G. B., Wakabayashi, N., Saunders, B., Shen, Y., Fujimura, T., Su, L. K. and Levin, B. (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N. Engl. J. Med. 342, 1946 – 1952.
170 Fujiki, H. and Suganuma, M. (1994) Tumor necrosis factor-alpha, a new tumor promoter, engendered by biochemical studies of okadaic acid. J. Biochem. (Tokyo) 115, 1 – 5.
171 Fujiki, H., Suganuma, M., Okabe, S., Sueoka, E., Suga, K., Imai, K. and Nakachi, K. (2000) A new concept of tumor promotion by tumor necrosis factor-alpha, and cancer preventive agents (−)-epigallocatechin gallate and green tea – a review. Cancer Detect. Prev. 24, 91 – 99.
172 Heiss, E., Herhaus, C., Klimo, K., Bartsch, H. and Gerhauser, C. (2001) Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J. Biol. Chem. 276, 32008 – 32015.
173 Konwinski, R. R., Haddad, R., Chun, J. A., Klenow, S., Larson, S. C., Haab, B. B. and Furge, L. L. (2004) Oltipraz, 3H-1,2-dithiole-3-thione, and sulforaphane induce overlapping and protective antioxidant responses in murine microglial cells. Toxicol. Lett. 153, 343 – 355.
174 Wierinckx, A., Breve, J., Mercier, D., Schultzberg, M., Drukarch, B. and Van Dam, A. M. (2005) Detoxication enzyme inducers modify cytokine production in rat mixed glial cells. J. Neuroimmunol. 166, 132 – 143.
175 Thejass, P. and Kuttan, G. (2006) Augmentation of natural killer cell and antibody-dependent cellular cytotoxicity in BALB/c mice by sulforaphane, a naturally occurring isothiocyanate from broccoli through enhanced production of cytokines IL-2 and IFN-gamma. Immunopharmacol. Immunotoxicol. 28, 443 – 457.
176 Killeen, M. E., Englert, J. A., Stolz, D. B., Song, M., Han, Y., Delude, R. L., Kellum, J. A. and Fink, M. P. (2006) The phase 2 enzyme inducers ethacrynic acid, DL-sulforaphane, and oltipraz inhibit lipopolysaccharide-induced high-mobility group box 1 secretion by RAW 264.7 cells. J. Pharmacol. Exp. Ther. 316, 1070 – 1079.
177 Chu, F. F., Esworthy, R. S. and Doroshow, J. H. (2004) Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. Free Radic. Biol. Med. 36, 1481 – 1495.
178 Mendelsohn, J., Howley, P. M., Israel, M. A. and Liotta, L. A. (Eds.) (2001) Molecular Basis of Cancer, 2nd edn, pp 173 – 185. London, Elsevier.
179 Pluda, J. M. (1997), Tumor-associated angiogenesis: mechanisus, clinical implications, and therapeutic strategies. Semin. Oncrl. 24, 203 – 218.
180 Bertl, E., Bartsch, H. and Gerhauser, C. (2006) Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. Mol. Cancer Ther. 5, 575 – 585.
181 Asakage, M., Tsuno, N. H., Kitayama, J., Tsuchiya, T., Yoneyama, S., Yamada, J., Okaji, Y., Kaisaki, S., Osada, T., Takahashi, K. and Nagawa, H. (2006) Sulforaphane induces inhibition of human umbilical vein endothelial cells proliferation by apoptosis. Angiogenesis 9, 83 – 91.
182 Thejass, P. and Kuttan, G. (2006) Antimetastatic activity of sulforaphane. Life Sci. 78, 3043 – 3050.
183 Kotlo, K. U., Yehiely, F., Efimova, E., Harasty, H., Hesabi, B., Shchors, K., Einat, P., Rozen, A., Berent, E. and Deiss, L. P. (2003) Nrf2 is an inhibitor of the Fas pathway as identified by Achilles' heel method, a new function-based approach to gene identification in human cells. Oncogene 22, 797 – 806.
184 Ohtsubo, T., Kamada, S., Mikami, T., Murakami, H. and Tsujimoto, Y. (1999) Identification of NRF2, a member of the NF-E2 family of transcription factors, as a substrate for caspase-3(-like) proteases. Cell Death Differ. 6, 865 – 872.
185 Wu, Q., Kirschmeier, P., Hockenberry, T., Yang, T. Y., Brassard, D. L., Wang, L., McClanahan, T., Black, S., Rizzi, G., Musco, M. L., Mirza, A. and Liu, S. (2002) Transcriptional regulation during p21WAF1/CIP1-induced apoptosis in human ovarian cancer cells. J. Biol. Chem. 277, 36329 – 36337.
186 Gartel, A. L. and Tyner, A. L. (2002) The role of the cyclin-dependent kinase inhibitor p21 in apoptosis. Mol. Cancer Ther. 1, 639 – 649.
187 Xu, C., Shen, G., Chen, C., Gelinas, C. and Kong, A. N. (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.

188 Jeong, W. S., Kim, I. W., Hu, R. and Kong, A. N. (2004) Modulation of AP-1 by natural chemopreventive compounds in human colon HT-29 cancer cell line. Pharm. Res. 21, 649 – 660.

189 Banning, A. and Brigelius-Flohe, R. (2005) NF-kappaB, Nrf2, and HO-1 interplay in redox-regulated VCAM-1 expression. Antioxid. Redox. Signal. 7, 889 – 899.

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