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Role of 4-hydroxynonenal in chemopreventive activities of sulforaphane

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Abstract

Chemoprevention of cancer *via* herbal and dietary supplements is a logical approach to combat cancer and presently it is an attractive area of research investigations. Over the years, the use of isothiocyanates, such as sulforaphane (SFN) found in cruciferous vegetables, has been advocated as chemopreventive agents and their efficacy has been demonstrated in cell lines and animal models. *In-vivo* studies with SFN suggest that besides protecting normal healthy cells from environmental carcinogens it also exhibits cytotoxicity and apoptotic effects against various cancer cell types. Among several mechanisms for the chemopreventive activity of SFN against chemical carcinogenesis, its effect on drug metabolizing enzymes that causes activation/ neutralization of carcinogenic metabolites is well established. Recent studies suggest that SFN exerts its selective cytotoxicity to cancer cells *via* reactive oxygen species (ROS)-mediated generation of lipid peroxidation (LPO) products particularly 4-hydroxynonenal (HNE). Against the background of the known biochemical effects of SFN on normal and cancer cells, in this article we have reviewed the underlying molecular mechanisms responsible for the overall chemopreventive effects of SFN focusing on the role of HNE in these mechanisms that may also contribute to its selective cytotoxicity to cancer cells.

Introduction

Cancer is one of the major causes of morbidity and mortality throughout the world. Carcinogenesis is a multistep molecular process induced by genetic and epigenetic changes that disrupt pathways controlling cell proliferation, apoptosis, differentiation, and senescence [1–4]. A major approach to fight against cancer is based on prevention of the disease through use of non-toxic dietary supplements, micronutrients, and natural products. This approach is generally referred to as chemoprevention that is defined as the use of natural or synthetic agents to inhibit, reverse, or prevent the development of cancer. The major goal of chemoprevention is to delay the onset of cancer as well as to decrease its incidence. Therefore, effective chemoprevention requires the use of non-toxic agents that

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inhibit specific molecular steps in the carcinogenic pathway. It has been advocated that vegetarian diet may be an important source of cancer-inhibiting bioactive phytochemicals. Although these phytochemicals are generally viewed as non-essential for normal body functioning, an increasing number of these agents have been shown to possess biological activities that are not only relevant for their ability to fight various diseases but also in the prevention of cancer [5–7]. In last couple of decades the efficacies of isothiocyanates, particularly those of sulforaphane isolated from the cruciferous vegetables, in cancer chemoprevention have been recognized and continue to be extensively studied for their pharmacological effects.

Occurrence and chemistry of isothiocyanates

There is epidemiological evidence suggesting that dietary intake of cruciferous vegetables may reduce the risk of different types of malignancies, including the prostate cancer [8–11]. The anticarcinogenic effect of these cruciferous vegetables including broccoli has been attributed to the abundance of isothiocyanates (ITCs) in these plants. ITCs occur naturally as the thioglucoside conjugates (glucosinolates) in a variety of edible plants including watercress, broccoli and cabbage etc. Various ITCs can be released from the hydrolysis of their respective glucosinolates through catalytic action of myrosinase [Fig. 1]. For example, the principal glucosinolate present in broccoli is glucoraphanin, which is hydrolyzed by myrosinase to yield SFN. It has been shown [12] that the hydrolysis of glucosinolates by myrosinase is influenced by the pH. While at neutral pH, ITC is the dominant product, acidic pH lead to an enhanced formation of nitrile derivatives [Fig. 1]. Additionally, presence of ferrous ions (Fe^{2+}) and epithiospecifier (ESP) protein also promotes the formation of nitrile during hydrolysis of glucosinolates and glucoaphanin respectively [13]. Most of the naturally occurring ITCs, including SFN, phenethyl-ITC (PEITC), and benzyl-ITC (BITC), have been shown to offer significant protection against cancer in animal models induced by a variety of chemicals including tobacco smoke-derived carcinogens [14,15]. It has been suggested that chemopreventive effects of different cruciferous plants may be influenced by the predominance of ITCs having characteristic side chains [14] such as methylsulfinyl-, benzyl-, 2-phenethyl-, methylthiopropyl and allyl groups [Table 1].

Chemopreventive effects of Sulforaphane (SFN)

In vivo studies

Among the ITCs listed in Table 1, SFN has been studied more widely and is shown to provide significant protection against chemical carcinogenesis in rodent models [16–20]. For example, incidence, progression, and severity of dimethylbenz(a)anthracene (DMBA)-induced mammary tumors has been shown to be significantly reduced in mice pretreated with the extracts of broccoli sprouts [11, 21]. SFN isolated from broccoli also inhibited DMBA-induced preneoplastic lesions in mouse mammary glands, rat mammary tumors, benzo[a]pyrene (BaP)-induced fore stomach tumors in mice, and inhibited proliferation of human breast cancer cells by down regulating the expression of estrogen receptor α [22–25]. SFN effectively reduced the formation of colonic aberrant crypt foci in azoxymethane (AOM) treated rats and suppressed the growth of intestinal polyps in mice [26]. SFN has also been shown to inhibit skin tumor genesis by acting prior to its initiation stage in mice and also retard the growth of PC-3 human prostate cancer xenografts in nude mice [27]. Furthermore, it has been shown that SFN-mediated oxidative stress can activate pro-apoptotic signaling in cancer cells that may inhibit cancer progression [27, 28].

In vitro studies

In vitro studies suggest that SFN may selectively inhibit proliferation of cancer cells by targeting the factors that provide advantage to growth and motility of cancer cells. For

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example, hypoxia-inducible factor-1α (HIF-1α) protein often constitutively expressed in cancer cells and is believed to provide advantages to their growth and motility [29, 30], is targeted by SFN. Studies on the effect of SFN on oral carcinoma cell lines *in vitro* have shown that HIF-1α is down-regulated by SFN treatment in the human tongue squamous carcinoma cell line, Tca8113 [31]. It has been reported that SFN causes the enhancement of apoptosis through the inhibition of cyclooxygenase-2 expression and NFκB-DNA binding in the human bladder T24 cell line [32]. SFN was first thought to play only a blocking role in the prevention of carcinogenesis by inducing enzymes that are critical to the removal of carcinogens. Now there is overwhelming evidence that SFN suppresses tumor progression in all stages, including metastasis [32–40]. Studies in animal models as well as *in vitro* systems have provided evidence of the anti-metastatic activities of SFN for different types of carcinomas [33–36]. The mechanisms of these effects of SFN have not been investigated thoroughly. More recent studies demonstrate that SFN is capable of inhibiting angiogenesis, metastasis, neovascularization, pro-angiogenic signaling, basement membrane integrity and endothelial cell migration, and tube formation [37, 38]. These effects were associated with transcriptional down-regulation of vascular endothelial growth factor (VEGF), HIF-1α, c-Myc, and matrix metalloproteinase-2 (MMP-2). SFN also inhibited the proliferation and tubular formation of human umbilical vein endothelial cells on matrigel *in vitro*, and was responsible for suppression of MMP-9 activity and invasiveness of human MDA-MB-231 breast cancer cells [35–40]. SFN specifically targets cancer cells and prevent their proliferation. While these studies are consistent with the known chemopreventive effects of SFN, these findings also provide insight into the underlying mechanisms responsible for the selective cytotoxicity of SFN to cancer cells. It is possible that while SFN-mediated signaling for the induction of defense mechanisms such as those associated with Nrf2, HSF1, NFkB may contribute to the protective effect of SFN to normal cells from carcinogenic insult, SFN may also activate pro-apoptotic signaling specifically in cancer cells. Possible mechanisms that may be involved in chemopreventive functions of SFN are briefly discussed in the following sections.

Mechanisms of chemoprevention

It was earlier believed that chemo preventive agents primarily inhibited chemical carcinogenesis by affecting the biotransformation enzymes of Phase I and Phase II and limiting the concentration of the ultimate carcinogens generated from procarcinogens. For example, the concentration of carcinogenic diol-epoxides generated from B(a)P can be minimized by inhibiting Phase I enzymes or by inducing Phase II enzymes [11,41]. There is ample evidence suggesting that SFN exerts at least some of its chemoprotective effects through the modulation of biotransformation enzymes. In addition, it can inhibit the development and proliferation of cancer cells by targeting various signaling pathways and the factors that provide advantage for the initiation, promotion, progression, and metastasis of cancer cells. The known biological activities of SFN are illustrated in Fig. 2.

Effect of SFN on biotransformation enzymes

Inhibition of Phase I cytochrome P450

It has been shown that SFN can modulate Phase I metabolism of xenobiotics through direct interactions with cytochrome P450 (CYP) enzymes or by regulating their transcript levels in cells. A dose dependent inhibition of CYP1A1 and CYP2B1/2 by SFN has been seen in rat hepatocytes. SFN has been shown to decrease the activity of CYP3A4 by suppressing its mRNA levels in human hepatocytes and there is additional indirect evidence indicating that SFN modulates the activity of various CYP enzymes [42–46]. Thus, SFN may at least partly exert its chempreventitive effect by protecting the normal cells from chemical carcinogenesis by inhibiting the activation of procarcinogens by cytochrome P450 enzymes.

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Activation of Phase II enzymes

Induction of Phase II detoxification enzymes is perhaps one of the major mechanisms through which many chemopreventive agents including SFN inhibit carcinogenesis [40, 47]. Over the past decade SFN has received much attention in cancer chemoprevention as it has been shown to be among the most potent naturally occurring inducers of Phase II enzymes, where a strong inverse relationship exists between tissue levels of these enzymes and susceptibility to chemical carcinogenesis. Phase II enzymes in general catalyze the conjugation of various metabolites generated in Phase I biotransformation to endogenous ligands such as GSH and glucuronic acid for their elimination [40, 47, 48]. However, this classification of Phase II enzymes is further expanded to include enzymes that catalyze a wide variety of reactions to provide protection against the toxicity of various electrophiles and reactive oxygen species (ROS) [41, 49–51]. ITCs form conjugates with GSH (GS-ITCs) that are subsequently metabolized to mercapturic acids (N-acetyl cysteine conjugates of corresponding ITC) to be excreted in the urine. Similar to other xenobiotic substrates of GSTs, various ITCs also induce GSTs to varying degrees and the accelerated detoxification of electrophilic carcinogens has been suggested to be one of the major mechanisms for their protective effect against chemical carcinogens [49–53].

Besides, glutathione S-transferases (GSTs), and UDP- glucuronosyl transferases (UGTs), SFN is also a very potent inducer of quinone reductase (NAD[P]H): quinone oxidoreductase (NQO) [52]. The induction of Phase II gene expression and enzyme activity by SFN has been shown in a number of model cell lines of different origin, the most commonly utilized being those derived from liver hepatoma, human HepG2, and mouse Hepa1c1c7 [53–56]. SFN and its GSH-conjugates have been shown to promote a significant increase in both UGT1A1 and GSTA1 mRNA levels in HepG2 and HT29 cells. Up to three fold induction of NQO1 has been reported in Hepa1c1c7 cells exposed to increasing levels of SFN for 24h [57]. The enzymatic activities of GST, NQO1, aldo-keto reductase (AKR), and glutathione reductase (GR) in various cancer cell lines (HepG2, MCF7, MDA-MB-231, LNCaP, HeLa and HT-29) are increased by 11 to 17 fold by non-toxic doses of SFN [58–61]. In another study SFN has been shown to significantly induce the activity and expression of Phase II enzymes in the human prostate cell lines LNCaP, MDA PCa 2a, MDA PCa 2b, PC-3 and TSU-Pr1[49]. SFN caused a robust and sustained transcriptional induction of NQO1 gene expression in these cells that was accompanied by an increase in corresponding enzymatic activity. More recently, induction of GSTs and NQO1 has also been reported in cultured bladder cancer cells [52]. Induction of NQO1 may be particularly important to the mechanisms through which SFN provides protection to neighboring normal cells from oxidative stress.

Activation of Phase II enzymes by SFN is not only in cancer cells but also in their normal counterpart and non-transformed cell lines. For example, highly induced levels of NQO1 protein have been detected in the non-transformed rat RL34 epithelial cell line. SFN also induces expression of GST A1-1, A2-2 isoforms and NQO1 in primary rat hepatocytes in a dose and time-dependent manner, although prolonged treatment is required to obtain GST induction levels comparable to those obtained in hepatoma cell lines [43]. Similar results have been reported in primary cultures of freshly isolated human hepatocytes where NQO1 gene expression was induced by SFN without any significant effect on GSTA1 transcription. It has also been reported that SFN induces UDPG1A1 and GSTA1 mRNA expression in human hepatocytes, although UGT1A1 induction was found to be a matter of inter-individual variation [43, 53]. Similar to other ITCs, SFN also causes the induction of Phase II enzymes *in vivo*. Increased Phase II enzyme activities have been observed in the liver, lung, mammary gland, pancreas, stomach, small intestine and colon of rats and mice treated with SFN [22–28, 40, 42–52]. These studies suggest that SFN-mediated induction of Phase II enzymes not only attenuates the levels of activated carcinogens but also provides

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protection against oxidative stress and these effects collectively contribute to its chemopreventive activity. It must be realized that induction of phase II enzymes should also offer protection against electrophilic stress to cancer cells that would be a deterrent to chemotherapy due to the accelerated detoxification of electrophilic chemotherapeutic agents. In fact many drug resistant cancer cell line over express GST isozymes, particularly GSTP1-1. Thus the selective cytotoxicity of SFN to various cancer cells reported in recent studies appears to be independent of its effect on biotransformation enzymes. As mentioned above and elaborated later in article, SFN seems to exert its selective toxicity to cancer cells by targeting genes/proteins that provide growth advantage to cancer cells and it is likely that the oxidative stress induced by SFN *via* the generation of ROS plays a major role in these mechanisms.

Role of Reactive Oxygen Species (ROS) in chemopreventive activity of SFN

Oxidative stress is a cellular imbalance between production and elimination of ROS and accumulation of these species has been implicated in several mammalian patho-physiologies [62, 63]. It is well established that exogenous or endogenous electrophilic compounds induce oxidative stress in aerobic organisms because of the generation of ROS and reactive nitrogen species. It has been shown that the treatment of cells with purified SFN results in the generation of ROS and induction of ROS-mediated signaling that may contribute to at least some of its chemopreventive properties [63–65]. Recent studies have shown that the SFN-induced generation of ROS in U937 cells was evident as early as 2h after treatment, and that it caused the loss of mitochondrial membrane potential (MMP) suggesting that ROS-transduced signaling for apoptosis may be responsible for SFN-induced cell death [66]. These findings were further validated by quenching of ROS generation with N-acetylcysteine, which not only prevented ROS generation but also conferred near-complete protection against SFN-induced MMP disruption, and apoptosis [66]. Recent investigations suggest that damaged mitochondria stimulate increase in ROS, with subsequent activation of signaling pathways that control cancer cell growth. SFN treatment also leads to an increased ratio of Bax/Bcl2, release of cytochrome C, and subsequent activation of caspase3 in these cells further suggesting that ROS-mediated loss of MMP contributes to the activation of apoptotic signaling in cancer cell types [27, 66, 67]. These studies strongly suggest a role of ROS in SFN-induced signaling that may be relevant to its chemopreventive properties and its selective toxicity to cancer cells. For example, it has been shown that SFN induces cell cycle arrest, and apoptosis in cancer cells (LnCap, PC3) but not in normal cells (Pr-Ec). Likewise, SFN is reported to inhibit growth, activate apoptosis, up regulate HDAC activity, and suppress expression of key proteins involved in breast cancer progression. Thus ROS may play a dual role in chemoprotective activity of SFN by protecting normal cells from electrophilic stress through induction of defense mechanisms and also specifically inhibiting the growth and proliferation of cancer cells. As discussed later in this review, our recent studies demonstrate that many of the apoptotic signaling effects of SFN described above can be abrogated by inhibiting SFN-induced LPO and accumulation of HNE in cells indicating a major role of HNE in the mechanisms of the biological activity of SFN [68].

Role of Nrf2 in the SFN-induced chemoprevention

The transcription of ARE-driven genes is regulated, at least in part, by the nuclear factor (erythroid derived 2)-like 2 (Nrf2) which, under normal conditions, is sequestered in cytoplasm by Kelch-like ECH associated protein 1 (Keap1) [69–72]. Upon exposure of cells to inducers of oxidative stress and certain chemopreventive agents such as SFN, Nrf2 dissociates from Keap1, translocates to the nucleus, binds to antioxidant response element(s) (AREs), and transactivates Phase II detoxifying and antioxidant genes[70]. Consistent with the known induction of Phase II enzymes by SFN, many of the Nrf2-dependent genes were found to be SFN inducible as indicated by the results of comparative transcriptional profiles

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of the small intestine of Nrf2 (+/ +) and Nrf2 (−/−) female mice treated with SFN. Analysis of these gene expression profiles identified as many as 26 genes whose expression was Nrf2-dependent. These SFN-inducible genes included not only xenobiotic-metabolizing enzymes such as GSTs, but also GSH biosynthesizing and NADPH-generating enzymes [70, 72] that are crucial for defense against oxidative stress. This may suggest that many of the reported beneficial effects of SFN particularly in normal cells may be due to the induction of defense mechanisms against oxidative stress. Recently, several clusters of genes dependent on Nrf2 for their expression have been identified in the liver of SFN-fed wild-type and Nrf2-deficient mice by using gene chip microarrays [73]. The products of genes induced by SFN through an Nrf2-dependent pathway were classified as xenobiotic-metabolizing enzymes, antioxidants, ubiquitin/proteasome systems, stress response proteins, kinases and phosphatases, proteins related to immune response, cell adhesion, cell cycle and cell growth, metabolism, transport proteins and transcription factors [70, 73]. These findings suggest a much wider role of Nrf2 in the mechanisms of SFN activities besides the transcriptional activation of Phase II drug metabolizing and antioxidant enzymes. The effect of SFN on these signaling pathways associated with Nrf2 [70, 73] and its significance to the biological activities of SFN must be further investigated. In the context of oxidative stress, the activity of SFN clearly seems to be dichotomous. That is, while it causes ROS generation and oxidative stress in cells leading to the activation of pro-apoptotic signaling [27, 65], it simultaneously activates defense mechanisms e.g. Nrf2, and HSF1 for protection against oxidative stress and its own toxicity. The possibility of these dichotomous effects of SFN being responsible for its differential effects on normal and cancer cells should be explored. HNE generated during ROS-induced oxidative stress is also known to have such dichotomous effects [74–79]. HNE induces apoptosis in all cancer cell lines studied so far in our laboratory [79]. In addition HNE can also simultaneously activate the defense mechanisms against oxidative stress including Nrf2 and HSF1 in cell lines as well as in mice *in vivo* [74–76]. These studies together with our recent findings [68] that many of the signaling effects of SFN including apoptosis are blocked in cells transfected with GSTA1-1 or GSTA4-4, where formation and accumulation of HNE is inhibited, strongly suggest a key role of HNE in the mechanisms of SFN- induced signaling and chemoprevention [Fig. 3]. This is further elaborated in the following sections.

HNE mediated signaling and its relevance to cancer

SFN mediated generation of ROS induces membrane LPO and generation of HNE [68] that is an inevitable consequence of ROS-induced stress [78–87]. Also as discussed above, there is ample evidence that the electrophilic products of LPO including lipid hydroperoxides and α, β-unsaturated carbonyls particularly, HNE play a crucial role in stress-induced apoptotic signaling [74–79, 82–87]. In recent years, HNE has emerged as an important second messenger molecule involved in signaling for cell proliferation, cell cycle arrest, differentiation, apoptosis, and the regulation of the expression of a multitude of genes in cells of diverse origin [82–87]. HNE has also been shown to modulate survival and death signaling pathways in a concentration dependent manner by interacting with several signaling proteins [74–79, 82–85]. Our studies have shown that HNE induces Fas-mediated extrinsic, as well as p53-mediated intrinsic pathways of apoptosis in different cell types [74–76]. The relevance of HNE-mediated signaling to cancer is evident from numerous studies showing that HNE induces apoptosis in various cell types studied so far and that at low levels, it could also limit its own toxicity by activating mechanisms for cell survival [74–76]. This appears to be true for SFN as well [Fig. 3].

Many of the effects of HNE on cell signaling have been determined by regulating its intracellular levels by GSTs that play a major role in regulating the levels of HNE in cells [78,79,82–87]. Apart from catalyzing the conjugation of carcinogenic electrophiles to GSH,

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the alpha class GSTs, GSTA1-1 and GSTA2-2 attenuate LPO by catalyzing the GSH-dependent reduction of phospholipid hydroperoxides (PL-OOH) and fatty acid hydroperoxides (FA-OOH) through its Se-independent glutathione peroxidase (GPx) activity thereby terminating the autocatalytic chain of LPO reactions resulting in decreased formation of HNE [86,87]. In addition, the isozyme GSTA4-4 can limit HNE levels in cells by catalyzing its conjugation of GSH in a highly efficient manner. A multitude of studies have demonstrated that HNE concentration in mammalian cells is regulated by a coordinated action of GSTA4-4 that conjugates HNE to GSH and RLIP76 that transports the conjugate, GS-HNE, out of cells [82–90]. It has been shown that elevated HNE levels in cells cause apoptosis and cancer cells can evade apoptosis by the up regulation of GSTs and RLIP76 and consequent lowering of HNE levels. The inhibition of RLIP76-mediated ATP-dependent transport of GS-HNE results in apoptosis in all cancer cell types studied so far in our laboratory [91–94]. More importantly, complete and sustained remission of the xenografts of melanoma, colon, lung, kidney and pancreas xenografts in nude mice has been demonstrated by blocking the transport of GS-HNE that leads to an increase in HNE concentrations in cells [91–94]. These rather remarkable findings further underscore the significance of HNE to cancer. HNE can promote survival pathways at low concentrations (sub-physiologic) and at higher (supra-physiologic) concentrations it promotes apoptosis via multiple pathways. Available evidence from a multitude of studies suggests that a basal constitutive level of HNE may be required for the normal cellular processes [79, 84]. It has been suggested that under the conditions of oxidative stress when concentration of HNE rise above this basal constitutive window it induces the pro-apoptotic signaling, while depletion of HNE to levels below this physiological window promotes proliferation and transformation [84].

Overlap in SFN and HNE-induced signaling

Similar to SFN, HNE has also been shown to induce stress-responsive pro- survival factors, such as Nrf2, heat shock factor 1 (HSF1) and its client heat shock proteins, and EGFR, and the transcription repressor Daxx, that can inhibit Fas mediated apoptosis [74–76, 82–86]. Some of the biological activities of SFN that are shared by HNE are listed in Table 2. Generation of HNE upon SFN exposure could therefore contribute to its protective effects against cancer through two mechanisms. First, it can activate the survival mechanisms to protect normal cells and second, it can promote apoptosis in cancer cells to inhibit their proliferation. In this model initially generated low levels of HNE may induce survival mechanisms beneficial for the normal cells but sustained oxidative stress caused by SFN and increase in HNE levels may selectively kill cancer cells and prevent their proliferation by targeting the factors that provide advantage to cancer cells in their proliferation. Our recent studies with human erythroleukemic cells are in line with this idea and show that indeed some of the biological activities associated with the chemoprotective properties of SFN are mediated through HNE generated during SFN-induced oxidative stress, and that these activities of SFN could be inhibited by the over expression of alpha class GST isozymes that attenuate HNE levels in cells [77]. For example, SFN induced cytotoxicity, cell cycle arrest, and apoptosis in HL60 and K562 cells is inhibited by forced over-expression of GSTA1-1 in these cells due to the attenuation of LPO and suppression of intracellular HNE. The idea of the biological activity of SFN being mediated via HNE finds support in many studies showing that HNE is a common denominator in the mechanisms of ROS-mediated signaling [68, 70]. HNE *per se* is known to cause cell cycle arrest, apoptosis, and cytotoxicity to cells via necrosis [78, 79, 95], the effects that are common with SFN. The observed effects of GSTA1-1 over expression on inhibition of the biological activities of SFN associated with the suppression of HNE levels [68] strongly implicate HNE in the mechanisms of chemopreventive effects of SFN. While it is possible that the increased SFN conjugating activity of GSTA1-1-overexpressing cells may lower the actual concentration of SFN by its

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accelerated conjugation with GSH, no significant alteration in the GSH levels of SFN-treated empty vector and hGSTA1-transfected cells was observed in these studies [68] suggesting that the protective effect of GSTA1-1 against SFN toxicity was preferentially imparted through the inhibition of SFN-induced LPO and consequent lowering of HNE levels, rather than GST-GSH-mediated detoxification of SFN.

The effects of SFN similar to those in HL60 and K562 cells have also been previously reported with colon and prostate cancer cells [65, 66, 96]. These effects include the induction of cell cycle arrest and apoptosis. It has been shown that SFN can arrest cell cycle at different stages of its progression, a mechanism by which it can inhibit growth of cancer cells. Arrest of cells in G0/G1, G2/M and S phases upon treatment with SFN have been reported in breast, bladder, colon and prostate cancer [27, 97–102]. A number of mechanisms have been proposed for the SFN-induced cell cycle arrest in different cell types. Cyclins and cyclin-dependent kinase complexes play an important role in the mechanisms of cell cycle progression [103,104]. By binding to Cdk1/2, cyclin B1 can activate Cdk1/2 (cdc2) to facilitate its nuclear accumulation for mitotic initiation in the late G2 phase of mammalian cells. It has been suggested that while SFN-induced cell cycle arrest in the G2/M phase appears to be regulated by cell cycle-related proteins cyclin B1 and Cdk1, the arrest in G1 phase is mediated by the inhibition of cyclin D1 and DNA synthesis [105–107]. Another suggested mechanism through which SFN induces cell cycle arrest is *via* the up regulation of CDKI such as p21 and p27 [65, 66, and 68]. Additionally, the SFN –induced cell cycle arrest has also been attributed to the disruption of normal mitotic microtubule polymerization and histone acetylation [108,109]. As summarized in Table 2, HNE shares many of these biological activities of SFN. Interestingly, many of these effects of SFN on cell cycle progression can be attenuated by over expression of GSTA1-1 or GSTA4-4 in cells [68]. HNE may be a causative factor for SFN-induced apoptosis *via* mitochondrial apoptotic pathways because in GSTA1-1-overexpressing cells SFN fails to induce apoptosis and unlike the wild type cells, HNE levels do not increase in these cells upon SFN treatment. In GSTA1-1-overexpressing cells, SFN-induced translocation of Bax to mitochondria, a pro apoptotic signal, is inhibited and anti-apoptotic signaling is activated as indicated by activation of Bcl-xL [68]. Furthermore, in GSTA1-1-overexpressing cells, the release of SFN-induced cytochrome c to the cytosol and nuclear accumulation of AIF is also inhibited. These studies suggest that caspase3 independent apoptosis by SFN is also HNE dependent that would further indicate a role of HNE in the biological activities of SFN [68]. Thus, at least some of the chemopreventive properties of SFN appear to be associated with generation of ROS and the accumulation of HNE in cells.

Both, SFN and HNE promote nuclear translocation of HSF1 and induction of the expression of Hsp70. SFN-induced up regulation of heat shock proteins [68] most likely results from the reverse nuclear-cytoplasmic trafficking of the transcription factor HSF1, its repressor protein Daxx. It has been shown that HNE also induces the translocation of Daxx from nucleus to cytoplasm and that of HSF1 from cytoplasm to nucleus [74]. Likewise both, HNE and SFN induce nuclear translocation and activation of Nrf2. SFN induced nuclear translocation of Nrf2 is more pronounced in GSTA1-1 over expressing HL60 and K562 cells as compared to empty vector transfected cells [68]. If HNE is the causative factor for such translocation, one may expect lesser nuclear translocation of Nrf2 and HSF1 in SFN-treated GSTA1-1 over expressing cells. This apparent anomaly could perhaps be due to the concentration dependent opposite effects of HNE on survival signaling discussed above. It is possible that initial low levels of HNE generated during SFN exposure act as a sensor to induce translocation of Nrf2 and HSF1 in both the vector and GSTA1-1 over expressing cells as a survival mechanism. But whereas in GSTA1-1 over expressing cells, low levels of HNE that are required for the translocation are maintained, in vector transfected cells sustained higher accumulation of HNE leads to apoptosis and apparently a lesser nuclear

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accumulation of Nrf2 and HSF1. This postulate however remains to be confirmed through further studies and the constitutive levels of HNE in cells that would promote either proliferation or cell death need to be clearly established. Thus available evidence strongly suggests that perhaps HNE plays a crucial role in the mechanisms of the biological activities of SFN including its chemoprotective properties i.e., protection of normal cells against oxidative/electrophilic stress by up regulating defense mechanisms, and specific killing of cancer cells by targeting signaling of pathways that provide selective growth advantage to cancer cells. Further studies to validate this conjecture may help in developing novel approaches for the search of effective chemoprotective agents.

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Highlights

- Sulforaphane present in cruciferous vegetables is a potent anti cancer agent.
- SFN can protect from chemical carcinogenesis and can selectively kill cancer cells.
- Toxicity of SFN is due to Reactive oxygen species mediated lipid peroxidation.
- 4 Hydroxynonenal (HNE) plays a major role in anti cancer activity of SFN.

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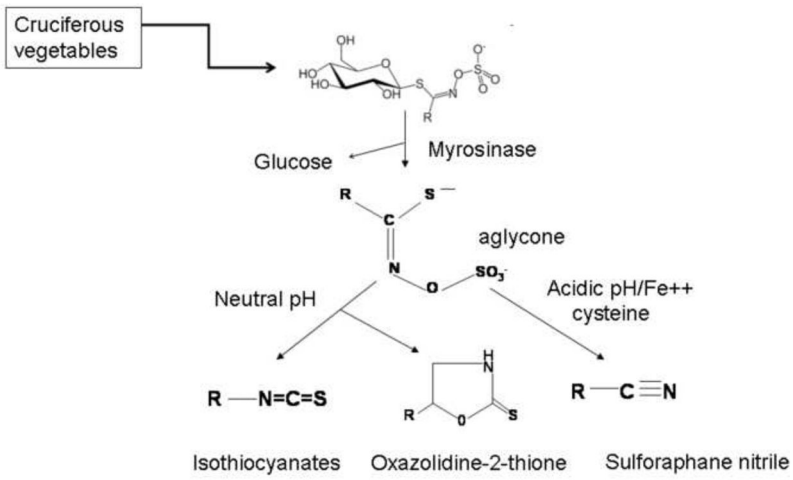

Figure 1.
Chemistry of naturally occurring isothiocyanates.

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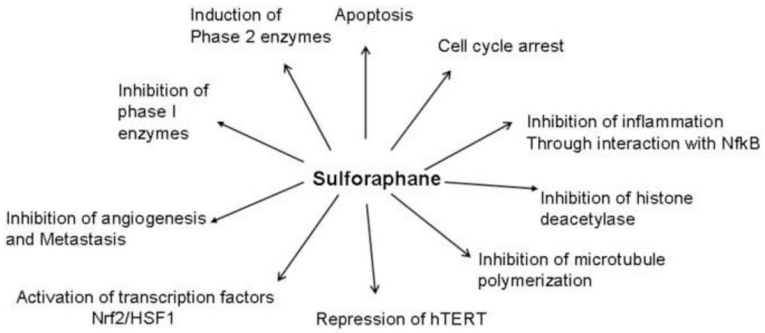

Figure 2.
Biological activities of Sulforaphane.

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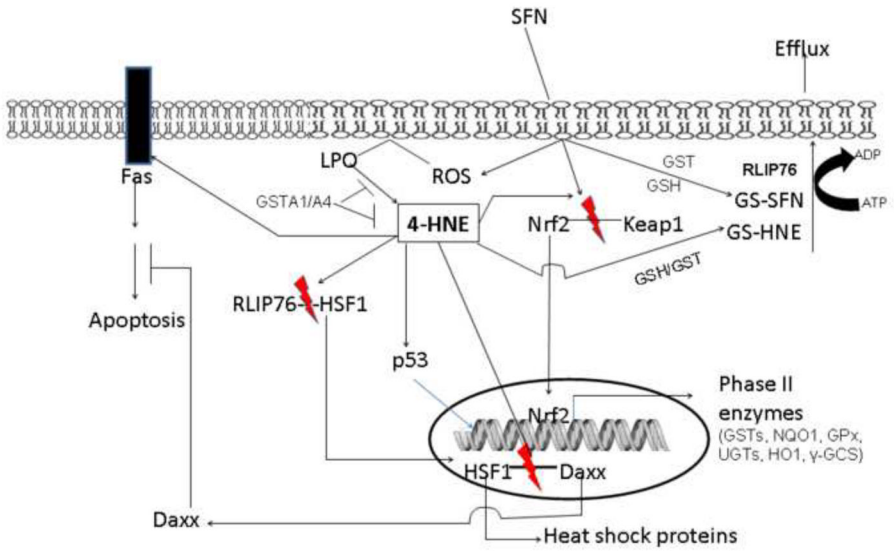

Figure 3.

ROS generated on exposure of SFN induces LPO [68] that leads to HNE formation. In turn, HNE causes activation and nuclear translocation of Nrf2, HSF1 and p53 [73–75] leading to enhanced transcription of genes associated with these transcription factors. Many of these genes contribute to the pro-survival pathways by inducing defense mechanisms against oxidative stress and enhanced detoxification of activated carcinogens/toxicants. Simultaneously, HNE can induce apoptosis in cancer cells through multiple pathways that may be self-regulated by HNE-mediated translocation of the transcription repressor Daxx which inhibits Fas-mediated apoptosis to protect neighboring cells from “run away” apoptosis. Thus SFN seems to provide protection against cancer by interrupting the initiation of cancer mediated by accelerating the detoxification of potential carcinogens as well as by killing cancer cells to prevent its progression.

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Table 1

Isothiocyantes present in cruciferous vegetables

Chemical Name	Structure	Vegetables
4-methylsulfinylbutyl isothiocyanate (SFN)	CH_3-S-CH_2-CH_2-CH_2-CH_2 N=C=S	Broccoli, Cauliflower
Benzyl isothiocyanate (BITC)	C6H5-CH2-N=C=S	Lepidium Cress
2-Phenethyl isothiocyanate (PIETC)	C6H5-CH2-CH2-N=C=S	Water cress, Radishes, turnips
3-Methylthiopropyl isothiocyanate	CH3-S-CH2-CH2-CH2-N=C=S	Cabbage
4-Methylthiobutyl isothiocyanate	CH3-S-CH2-CH2-CH2-CH2-N=C=S	Arugula
2-Propenyl isothiocyanate (AITC)	CH2=CH-CH2-N=C=S	Mustard, Cabbage, Cauliflower
4-methylsulfinylbutyl isothiocyanate (SFN)	CH3-S-CH2-CH2-CH2-CH2 N=C=S	Broccoli, Cauliflower
Benzyl isothiocyanate (BITC)	C6H5-CH2-N=C=S	Lepidium Cress
2-Phenethyl isothiocyanate (PIETC)	C6H5-CH2-CH2-N=C=S	Water cress, Radishes, turnips
3-Methylthiopropyl isothiocyanate	CH3-S-CH2-CH2-CH2-N=C=S	Cabbage
4-Methylthiobutyl isothiocyanate	CH3-S-CH2-CH2-CH2-CH2-N=C=S	Arugula
2-Propenyl isothiocyanate (AITC)	CH2=CH-CH2-N=C=S	Mustard, Cabbage, Cauliflower

* Compiled and modified from references [21, 34, 139]

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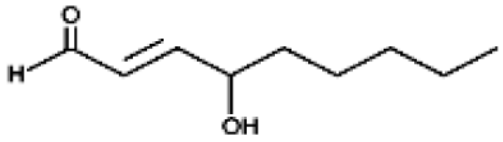
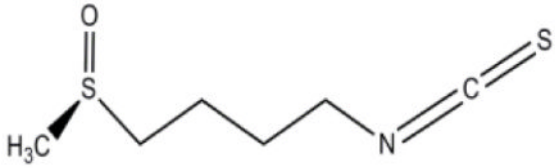
Table 2

Some of the common biological effects of HNE and SFN.

	Biological Effects	References for HNE 	References for SFN 
1	Apoptosis	74–77,82–87, 113.	51, 63–66, 68, 97, 110–112, 119.
2	Induction of Bax, p21, Caspase3	75,79, 136.	66, 135, 137.
3	Loss of Membrane Potential.	138.	65, 66, 137.
4	Cell Cycle Arrest	113,117, 130	51, 65, 97, 105–107, 110, 112.
5	Interaction with NfkB	123–126,129.	51, 116, 120.
6	Inhibition of HDAC	117, unpublished studies in our laboratory.	109, 110, 118.
7	Activation of Nrf2	121,126.	51, 73, 119, 120.
8	Activation of HSF1	122, 127.	68,128.
9	Activation of p53	74–76.	134.
10	Induction of HSP70	74, 122.	128.
11	Repression of hTERT	114, 131	115, 132.
12	Induction of oxidative stress	78,82–87, 130.	64–66,68
13	Activation of MAPK	125.	133.
14	Angiogenesis	83.	36–40.