Chemopreventive activity of sulforaphane

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Abstract: Cancer is one of the major causes of morbidity and mortality in the world. Carcinogenesis is a multistep process induced by genetic and epigenetic changes that disrupt pathways controlling cell proliferation, apoptosis, differentiation, and senescence. In this context, many bioactive dietary compounds from vegetables and fruits have been demonstrated to be effective in cancer prevention and intervention. Over the years, sulforaphane (SFN), found in cruciferous vegetables, has been shown to have chemopreventive activity in vitro and in vivo. SFN protects cells from environmental carcinogens and also induces growth arrest and/or apoptosis in various cancer cells. In this review, we will discuss several potential mechanisms of the chemopreventive activity of SFN, including regulation of Phase I and Phase II drug-metabolizing enzymes, cell cycle arrest, and induction of apoptosis, especially via regulation of signaling pathways such as Nrf2-Keap1 and NF-κB. Recent studies suggest that SFN can also affect the epigenetic control of key genes and greatly influence the initiation and progression of cancer. This research may provide a basis for the clinical use of SFN for cancer chemoprevention and enable us to design preventive strategies for cancer management, reduce cancer development and recurrence, and thus improve patient survival.

Keywords: sulforaphane, tumor, chemoprevention, Phase I and Phase II drug-metabolizing enzymes, apoptosis, anti-inflammatory, cell cycle progression, epigenetics

Introduction

Cancer is one of the major causes of morbidity and mortality in the world. Tumor development is a multistep process, including initiation, promotion, and progression, and involves genetic and epigenetic changes that disrupt pathways controlling cell proliferation, apoptosis, differentiation, and senescence.1-3

Cancer chemoprevention is defined as the use of dietary or pharmacological agents to prevent, block, or even reverse the process of tumor development before clinical manifestation of the disease.4 The major goal of chemoprevention is to delay the onset of cancer and to decrease its incidence. Therefore, effective chemoprevention requires the use of compounds that inhibit specific molecular steps in the carcinogenic pathway. These preventive measures that target the various steps involved in cancer initiation and progression could significantly decrease the incidence and mortality of cancer. In particular, the use of dietary chemoprevention strategies has gained significant interest.5 The use of chemopreventive compounds may have a significant impact on establishing recommendations for high-risk cancer patients, thereby increasing their survival through simple dietary choices with easily accessible foods.6 Some studies suggest that cruciferous vegetable intake may lower overall cancer risk, including colon, lung, and prostate cancer, particularly during the early stages.4,5,7 This inverse relationship is strongest for the consumption of cruciferous vegetables, especially those of the Brassica genus (including Brussels sprouts, broccoli, cabbage, cauliflower, and bokchoy).4 Thus, there is a growing interest in identifying specific chemoprotective...
 constituents in cruciferous vegetables and their mechanisms of action. Sulforaphane (SFN), which is converted from a major glucosinolate in broccoli/broccoli sprouts, has been shown to prevent chemically induced cancers in animal models and to inhibit the growth of established tumors.

The mechanisms whereby SFN exerts chemopreventive activity include modulation of Phase I and II xenobiotic-metabolizing enzymes and direct inhibition of binding of carcinogens to DNA. As a result, SFN inhibits DNA adduct formation and reduces the mutation rate. SFN also has anti-inflammatory effects, thereby preventing inflammation-mediated tumor formation by regulating the secretion of tumor necrosis factor alpha, IL-1β, IL-6, interferon gamma, IL-2, and IL-10. In addition, SFN can arrest cell cycle progression, particularly in the G2/M phase, and high concentrations of SFN can activate proapoptotic pathways.

Recent studies suggest that SFN can also regulate the epigenetic control of key genes including CDKs, p21, Bax, and Nrf2, and thus greatly influence the initiation and progression of cancer.

There is growing experimental evidence to support the efficacy of SFN in regulating the prevention and treatment of cancer through several different mechanisms. The aim of this review is to summarize the chemopreventive activity of SFN.

The characteristics and biological activity of SFN

Isothiocyanates (ITCs), plant-derived chemoprotective constituents, are formed by the hydrolysis of their precursor parent compounds, glucosinolates. The levels of glucosinolate vary greatly within members of the Cruciferae family, depending on the environment and genotype. SFN is an important and well-studied ITC derived from cruciferous vegetables, including broccoli, cauliflower, cabbage, and kale, with the highest concentration found in broccoli and broccoli sprouts.

SFN has antioxidant, antiproliferative, and anticarcinogenic properties. The epidemiological surveys in the US, Europe, China, and Singapore have established the associations between consumption of cruciferous vegetables and the reduction of carcinogenic risk. Numerous experimental studies also confirm the preventive effect of SFN in chemically induced lung, breast, renal, prostate, and colon cancers.

There are three key stages in the development of cancer: initiation, maintenance, and progression. SFN can inhibit the initiation of tumor development or halt the progression of cancer. Prevention of cancer initiation can be achieved by minimizing the exposure of cells to environmental carcinogenic factors through inhibition of their activation or by promoting their detoxification. In addition, SFN can also exhibit chemopreventive behavior by interfering with various signaling pathways that regulate oxidative stress, inflammation, cell proliferation, differentiation, and apoptosis. Thus, SFN can impact all stages of tumor development.

In rats, the pharmacokinetics of SFN was assessed following an oral dose of 50 μmol of SFN. The plasma concentration of SFN can be detected at 1 hour and it peaks at 20 μM at 4 hours. The concentration of SFN in plasma increases with the activation of various clusters of genes that are important in cellular defense mechanisms and cell cycle regulation, such as metallothionein, GSTA3, and MAPK in rat livers. It has been reported that glucosinolate or ITC preparations (the main components of SFN) were administered to the rats with dimethylbenzanthracene-induced mammary tumors by daily gavage. The mammary tumor development was significantly retarded in terms of both tumor multiplicity and incidence.

SFN can also inhibit skin tumorigenesis in mice and inhibits the growth of PC-3 cell xenografts in nude mice. In addition, the ability of SFN to inhibit tumor growth, metastasis, and angiogenesis and to enhance the therapeutic potential of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) suggests that SFN alone or in combination with TRAIL can be used for the management of embryonal rhabdomyosarcomas.

Traditionally, the major mechanism of protection against the initiation of carcinogenesis by SFN includes modulation of Phase I and Phase II xenobiotic-metabolizing enzymes that increase cell defenses against oxidative damage and promote the removal of carcinogens. However, there is ample evidence suggesting that SFN can have chemoprotective effects through multiple mechanisms, including the modulation of biotransformation enzymes, induction of apoptotic signaling pathways, arrest of cell cycle progression, as well as inhibition of angiogenesis and inflammation. In addition, recent studies suggest that SFN can also affect the epigenetic control of key genes and, thus, greatly influence the initiation and progression of cancer.

SFN inhibits Phase I and Phase II drug-metabolizing enzymes

Virtually, all carcinogens are exposed to metabolism when they enter the body. These metabolic processes mainly include oxidation, reduction, hydrolysis, and conjugation reactions, which cause the chemical carcinogens to become more hydrophilic. Phase I (cytochrome P450, CYP) drug-metabolizing enzymes (DMEs) are frequently involved...
in the metabolic processing of carcinogenic chemicals.\textsuperscript{24} The formation of electrophilic reactive metabolites or procarcinogens is often catalyzed by CYP DMEs through two electron oxidations to a hydroxylated or epoxidized medium.\textsuperscript{27} These chemical reaction processes have been defined as the bioactivation of carcinogens.\textsuperscript{2} Numerous studies have demonstrated that SFN can inhibit the DNA adduct and chemical carcinogenic processes through regulation of certain CYP isoforms via a competitive mechanism and a direct covalent modification in rodents.\textsuperscript{28} SFN alone did not significantly alter the activity and expression of the studied DMEs, except for GSTA1 at the mRNA level, which was significantly enhanced.\textsuperscript{29} However, SFN dose-dependently inhibited the activities of CYP1A1 and 2B1/2 in rat hepatocytes.\textsuperscript{29} In other reports, SFN also reduced CYP3A4 activity by decreasing CYP3A4 transcript level in rat hepatocytes.\textsuperscript{30} It has also been shown that SFN is a potent competitive inhibitor of CYP2E1 with a Ki of 37.0±4.5 mM in microsomes from livers of acetone-treated rats, and SFN is able to inhibit the genotoxicity of \textit{N}-nitrosodimethylamine.\textsuperscript{31} Overall, these findings suggest that SFN must be able to inhibit the activities of numerous CYPs and potentially alleviate the activation of procarcinogens. SFN has also been shown to effectively inhibit the chemically induced DNA adduct formation and tumorigenesis following exposure to benzo[a]pyrene and 1,6-dinitropyrene in the human mammary epithelial cell line MCF-10F.\textsuperscript{32} All these reports provide evidence that SFN protects against carcinogen-induced DNA damage, and support that the inhibition of Phase I enzymes plays a role in the chemopreventive activity of SFN.

Another major mechanism not involving the modulation of Phase I DMEs is the induction of Phase II DMEs, which transform carcinogens to inactive metabolites that are easily eliminated from the body, thus preventing their reaction with DNA. Over the past few decades, SFN has attracted a lot of attention in cancer chemoprevention since it is among the most potent naturally occurring inducers of Phase II enzymes, central to the processes of chemical carcinogenesis and other chronic degenerative diseases.\textsuperscript{41} A number of studies have revealed that the effects of SFN on Nrf2 and its downstream cytoprotective genes are through modification of Keap1 cysteine residues;\textsuperscript{42} activation of MAPK, PI3K, and PKB/Akt pathways; and epigenetic modifications, which result in the phosphorylation, nuclear accumulation, and increased transcription and stability of Nrf2.\textsuperscript{43,44} SFN can react with the thiol groups of Keap1 to form thionoacetyl adducts, which enables Nrf2 to escape Keap1-dependent ubiquitination and degradation, leading to nuclear localization of Nrf2.\textsuperscript{45,46} The nuclear Nrf2 binds to ARE and activates Phase II detoxifying and antioxidant genes.\textsuperscript{47} Shan et al reported that p38 MAPK activation could regulate Nrf2-ARE–driven enzymes, thereby facilitating the role of SFN in chemoprevention of bladder cancer.\textsuperscript{47} The expression and activity of HO-1 by SFN regulation has been shown to be Nrf2 dependent in mouse peritoneal macrophages.\textsuperscript{48} Also, activation of PI3K–PKB/Akt signaling regulated cell survival and Nrf2-driven HO-1 expression in SFN-treated human mesothelioma MSTO-211H cells.\textsuperscript{49} Wu et al also indicated that SFN singly or in combination with estrogen increased Nrf2 activity through activation of the PI3K pathway in breast cancer cell line MCF-7.\textsuperscript{50} SFN exerted an anticancer effect in a mouse skin tumor model, which involved the epigenetic reprogramming of Nrf2 leading to epigenetic reactivation of Nrf2 and subsequent induction of downstream target genes HO-1, NQO1, and UGT1A1.\textsuperscript{51}

Taken together, it appears that SFN, at least in part, inhibits tumor initiation by inhibiting Phase I enzymes and stimulating Phase II enzymes.
The anti-inflammatory activity of SFN

Chronic inflammation and carcinogenesis are thought to be closely related, and SFN has been found to have anti-inflammatory properties. Constitutive activation of NF-κB is common in various human malignancies, including breast and prostate cancer, and leads to the upregulation of genes encoding adhesion molecules, inflammatory cytokines, growth factors, and antiapoptotic genes.

NF-κB is a transcription factor that regulates the expression of several proinflammatory genes, most notably including nitric oxide, inducible nitric oxide synthase, Cox-2, and TNF-α. Elevated levels of Cox-2 have been monitored in various tumors and may account for excessive production of prostaglandin. In human malignant glioblastoma cells, SFN can also significantly decrease NF-κB expression compared to control cells, suggesting that NF-κB is an important molecular target of SFN.

SFN can reduce the DNA-binding activity of NF-κB directly by indirectly interacting with the thiol groups leading to dithiocarbamate formation and directly uniting with essential cysteine residues of NF-κB subunits, thereby reducing their DNA-binding capacity. In addition, SFN can also interact with glutathione and other redox regulators such as thioredoxin and Ref-1, which indirectly intervenes with NF-κB DNA-binding activity. These findings further confirm that thiol reactivity and redox modulation are important in the regulation of NF-κB-dependent transcription by SFN. Recently, the functions of SFN on natural killer cells and cell-mediated immune response were also researched in normal and tumor-bearing BALB/c mice, where administration of SFN significantly enhanced natural killer cell activity, antibody-dependent cellular cytotoxicity, and the production of IL-2 and interferon gamma in both normal as well as tumor-bearing mice.

Thus, the inactivation of NF-κB is an important chemopreventive mechanism of SFN. This suggests that SFN manifests anticarcinogenic effects not only through the regulation of biotransformation enzymes, but also by modulation of inflammation.

The apoptosis-inducing properties of SFN

There are various options by which cells are able to undergo cell death, and their respective mechanisms can be described based on their unique morphological features. Apoptosis, in contrast to other forms of cell death such as necrosis and autophagy, is the most prominent form of programmed cell death during animal development and maintenance of tissue homeostasis. Apoptosis is regarded as a “silent” mechanism of cell elimination projected to digest the contents of damaged cells and ensure the elimination of cells that are no longer necessary for the organism. Apoptosis is an essential function during the development and homeostasis of animals and cells, but inappropriate regulation of apoptosis may cause serious disorders. For example, excessive apoptosis has been related to neurodegenerative diseases, organ failure, autoimmune diseases, and cancers resulting from exposure to toxins. Many factors, including various genotoxic compounds and complicated environmental stresses, are responsible for the initiation and execution of apoptosis.

Data clearly show that SFN is a powerful inducer of apoptosis both in vitro and in vivo. Twenty years ago, the first evidence of SFN having proapoptotic activity was provided in a report of an antitumor drug in human colon cancer cells, where SFN could decrease the viability of HT29 and Caco-2 cells. Then, several other reports using in vitro models also demonstrated that SFN was able to mediate apoptosis by regulating multiple targets in the apoptotic pathway. SFN has been shown to target several steps involved in apoptosis, including downregulation of the expression of antiapoptotic factors Bcl-2 and Bcl-XL, upregulation of proapoptotic Bax, proteolytic activation of caspase-3, and degradation and/or cleavage of poly(ADP-ribose) polymerase. Additionally, it has been demonstrated that SFN can trigger the activation of Bax, downregulate inhibitor of apoptosis proteins (IAP) family (including cIAP1, cIAP2, and XIAP), and induce the activation of Apaf-1 in human prostate cancer cells.

The mechanisms whereby SFN regulates apoptosis are diverse. It was demonstrated that SFN-induced apoptosis is mediated by reactive oxygen species (ROS)-mediated activation of AMPK in human gastric cancer cells. Treatment of PC-3 cells with SFN can lead to ROS generation and break the mitochondrial membrane potential, which causes cytosolic release of cytochrome c and apoptosis. Apart from ROS, the MAPK pathway has also been reported to be activated by SFN in human prostate cancer PC-3 cells. SFN has also been shown to activate AP-1, which requires the activation of ERK and JNK signaling pathways, during the regulation of cell death elicited by SFN in PC-3 cells.

Mitochondrial signaling pathways play an important role in apoptosis, and SFN can activate the intrinsic/mitochondrial apoptotic pathway. This apoptosis involves release of cytochrome c from the mitochondria into the cytosol, which then binds Apaf-1 and leads to activation of the “apoptosis initiator” caspase-9. Furthermore, decreased mitochondrial
potential has been detected in prostate and bladder cancer cells in response to SFN.\textsuperscript{64,65} Apart from the activation of a caspase-dependent apoptotic pathway, SFN has also been shown to induce apoptosis in a caspase-independent manner. Subsequent release of mitochondrial proteins including cytochrome c, Smac, and AIF has also been observed. Treatment of glioblastoma cells with SFN (40 \textmu M) for 24 hours can cause a significant increase in the cytotoxic protein expression levels of AIF.\textsuperscript{66} All of the above evidence demonstrates that SFN can activate the mitochondrial apoptotic pathway.

A number of studies have also revealed that SFN can activate the death receptor/extrinsic pathway of apoptosis.\textsuperscript{57,66} Shankar et al demonstrated that SFN could upregulate the protein expressions of TRAIL receptor-1 (TRAIL-R1/DR4), TRAIL-R2/DR5, Bax, and Bak, and could inhibit the activation of NF-\kappaB, PI3K/AKT, and MEK/ERK pathways in prostate tumor tissues.\textsuperscript{69} However, the activation of the downstream effector caspase-3 has been well documented for SFN in many pathways.\textsuperscript{70,71}

SFN-induced apoptosis is also involved in the regulation of proteins in the IAP family, which can inhibit the activity of caspase signaling pathway. After treatment of prostate cancer PC-3 and LNCaP cells with SFN, the protein expression of all three members of the IAP family, including XIAP, c-IAP1, and c-IAP2, was significantly reduced.\textsuperscript{72} Furthermore, the expression of the IAP family members c-IAP1 and c-IAP2 was also downregulated with SFN treatment in human glioblastoma cells.\textsuperscript{66}

**The induction of cell cycle arrest of SFN**

The progression of the cell cycle through all four phases, G\textsubscript{1}, S, G\textsubscript{2}, and M, is adjusted by CDKs and cyclins.\textsuperscript{73} It has been shown that SFN can arrest the cell cycle at various stages of its progression, thereby inhibiting the growth of cancer cells. Induction of cell cycle arrest in G\textsubscript{0}/G\textsubscript{1}, S, and G\textsubscript{2}/M phases upon treatment with SFN has been reported in breast,\textsuperscript{74} bladder,\textsuperscript{65} colon,\textsuperscript{75} and prostate cancer cells.\textsuperscript{76,77} Arrest at the G\textsubscript{0}/G\textsubscript{1} phase of the cell cycle in response to SFN was associated with a higher depolarization of the mitochondrial membrane potential and intracellular ROS generation in human non-small-cell lung cancer cells,\textsuperscript{78} as well as a decreased phosphorylation of tumor suppressor RB and protection of the RB–E2F-1 complex in epithelial ovarian cancer cell lines of MDAH 2774 and SkOV-3.\textsuperscript{79} Another study demonstrated that G\textsubscript{2}/M phase arrest by SFN was achieved in human ovarian cancer cell line PA-1 via CDK1 downregulation and dissociation of the cyclin B1/CDK1 complex.\textsuperscript{80} However, the ability of SFN to specifically cause cytotoxicity in cancer cells, not normal cells, is an important factor in determining its safety and clinical relevance as a chemoprevention agent. It has been proved by Clarke et al’s team that SFN selectively induced cell cycle arrest and apoptosis in hyperplastic and cancerous prostate cells, but not PrEC normal cells.\textsuperscript{76} The differences in sensitivity to SFN in PrEC and cancerous prostate cells are likely due to a decrease in several class I HDAC proteins, induction of histone acetylation at the P21 promoter, and ultimately, induction of G\textsubscript{2}/M cell cycle arrest and apoptosis, and not due to differences in SFN metabolism or differences in Phase II enzyme induction.\textsuperscript{76}

Substantial evidence indicates that the concentration of SFN is responsible for these different effects. G\textsubscript{2}/M arrest was observed with treatment of SFN at a dose of 20 \textmu M, whereas concentrations of 100 \textmu M induced the accumulation of cells in the sub-G\textsubscript{1} phase, cell death, and dissipation of mitochondrial membrane potential in human colon adenocarcinoma cell Caco-2.\textsuperscript{81} SFN at 10 \textmu M can reduce cell viability and induce G\textsubscript{2}/M phase arrest in prostate cancer DU145 cells.\textsuperscript{82} Another G\textsubscript{2}/M phase arrest of SFN was observed in ovarian cancer PA-1 cells with 12.5 \textmu M SFN.\textsuperscript{80} Other evidence also indicates that the growth inhibitory effect of SFN is highly time dependent.\textsuperscript{83} Inhibition of cell growth by SFN followed a biphasic pattern in human colon cancer cells: transient exposure of human colon carcinoma cell line HT-29 to SFN for up to 6 hours resulted in reversible G\textsubscript{2}/M cell cycle arrest and cytostatic growth inhibition even at high concentrations.\textsuperscript{84} After a minimum continuous exposure time of 12 hours, SFN could irreversibly induce cell cycle arrest in the G\textsubscript{2}/M phase and the cells appeared to subsequently undergo apoptosis.\textsuperscript{84} These data indicate that SFN can influence cell cycle arrest through varying complex regulatory mechanisms.

**Epigenetic regulation of cancer genes by SFN**

Many natural dietary agents have been confirmed to be effective in cancer prevention. Treatment of cancer cells with these nutraceuticals often mediates favorable epigenetic changes.\textsuperscript{53} There are three common types of epigenetic mechanisms in mammals, including changes in DNA methylation, histone modifications, and non-coding RNAs.

DNA methylation is the most extensively researched of the epigenetic processes. DNMT and HDAC often work together in larger protein complexes to strip chromatin of active acetylation marks and lay down DNA methylation for stable gene repression. Enzymes that regulate the epigenetic signature of cancer cells have proven to be a viable target
in cancer prevention and cancer therapeutic research. Hsu et al confirmed that SFN could significantly decrease the expression of DNMTs and repressed methylation-silenced cyclin D2 in prostate cancer cells. These results demonstrate that SFN has the ability to epigenetically modulate cyclin D2 expression, and provide novel insights into the mechanisms by which SFN may regulate the gene expression as a chemopreventive agent in prostate cancer. Another paper also revealed that SFN can promote breast cancer cell death by adjusting the levels of DNMT and HDAC and downregulating the levels of cyclin D1, CDK4, and pRB.

Many cancers often exhibit aberrant patterns of histone modification and non-coding RNAs. SFN-induced suppression of tumors in Apc-minus mice was associated with inhibition of HDAC activity and concomitant increases in acetylation of histone H3 and H4 located in the promoter region of p21 and Bax genes. Zhang et al showed that SFN could increase mRNA and protein expressions of Nrf2 and its downstream target gene NQO1 by decreasing the protein levels of DNMT1 and DNMT3a. SFN also attenuated the protein expression levels of HDACs 1, 4, 5, and 7, while increasing the level of active chromatin marker acetyl-histone 3 (Ac-H3) during tumorigenesis in vivo (using TRAMP mice) and in vitro (using TRAMP C1 cells). These results suggest that SFN may exert its chemopreventive effect partly via epigenetic modifications, and via expression of the Nrf2 gene with subsequent induction of its downstream antioxidative stress pathway.

**Conclusion**

Human clinical studies have supported the chemopreventive effects of SFN on carcinogenesis. The clinical Phase I and Phase II studies showed that broccoli sprout extracts containing SFN were well tolerated and caused no significant adverse effects in healthy volunteers, women with breast cancer, and men with recurrent prostate cancer. In addition, a recent study reported that SFN effectively inhibited tumor growth and increased the sensitivity of cancer cells to chemotherapeutics in patients with advanced pancreatic ductal adenocarcinoma.

Based on the above-mentioned studies, it is clear that the SFN is a safe and relatively nontoxic chemopreventive agent, and exerts anticancer activities through multiple mechanisms, including regulation of Phase I and Phase II DMEs, anti-inflammatory activity, cell cycle arrest, induction of apoptosis, and the epigenetic regulation on Nrf2-Keap1, cyclins, and CDKs. Further understanding of the cancer chemopreventive activities of SFN will allow us to assess its efficacy in human cancers as a single agent or as part of combination strategies in various types of human cancers.

**Acknowledgments**

This study was supported in part by grants from the National Science Foundation of China (81570344 to Ying Xin), the Norman Bethne Program of Jilin University (2015225 to Ying Xin, 2015203 to Xin Jiang), the Fundamental Research Funds for the Jilin University (450060501512 to Xin Jiang), the Education Department of Jilin Province Foundations (2016–448 to Xin Jiang), and the Health and Family Planning Commission of Jilin Province Foundations (2016Q034 to Ying Xin and 2015Q010 to Xin Jiang).

**Disclosure**

The authors report no conflicts of interest in this work.

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