Chemopreventive activity of sulforaphane

Xin Jiang¹Ye Liu²Lixin Ma¹Rui Ji³Yaqin Qu¹Ying Xin⁴Guoyue Lv⁵

¹Department of Radiation Oncology,
The First Hospital of Jilin University,
Changchun 130021, China;

²Department of Pathobiology, The
Fifth Affiliated Hospital of Sun Yat-
Sen University, Zhuhai 519000, China;

³Department of Internal Medicine,
Florida Hospital, Orlando, FL, USA;

⁴Key Laboratory of Pathobiology,
Ministry of Education, Jilin University,
Changchun 130021, China;

⁵Department of General Surgery,
The First Hospital of Jilin University,
Changchun 130021, China

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- κ B. 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).⁸ Thus, there is a growing interest in identifying specific chemoprotective

Correspondence: Ying Xin
Key Laboratory of Pathobiology, Ministry
of Education, Jilin University, 126 Xinmin
Street, Changchun 130021, China
Tel +86 139 4491 4579
Email xinying13@126.com

Guoyue Lv
Department of General Surgery,
The First Hospital of Jilin University,
71 Xinmin Street, Changchun 130021,
China
Tel +86 136 8982 5113
Email lgy08@sina.com

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.⁹

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,¹⁰ thereby preventing inflammation-mediated tumor formation by regulating the secretion of tumor necrosis factor alpha, IL-1 β , IL-6, interferon gamma, IL-2, and IL-10. In addition, SFN can arrest cell cycle progression, particularly in the G₂/M phase,¹¹ and high concentrations of SFN can activate proapoptotic pathways.¹² 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.¹³

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.¹⁴ 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.¹⁴ SFN has antioxidant, antiproliferative, and anticarcinogenic properties.¹⁵ The epidemiological surveys in the US, Europe, China, and Singapore have established the associations between consumption of cruciferous vegetables and downtrend of carcinogenic risk.^{16,17} Numerous experimental studies also confirm the preventive effect of SFN in chemically induced lung, breast, renal, prostate, and colon cancers.^{8,9,11,18,19}

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.¹⁶ 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.^{2,17}

In rats, the pharmacokinetics of SFN was assessed following an oral dose of 50 μ mol of SFN. The plasma concentration of SFN can be detected at 1 hour and it peaks at 20 μ M 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.²⁰ 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.²¹ SFN can also inhibit skin tumorigenesis in mice and inhibits the growth of PC-3 cell xenografts in nude mice.^{22,23} 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.²⁴

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.²⁵ 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.^{13,26}

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.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.²⁷ These chemical reaction processes have been defined as the bioactivation of carcinogens.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.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.²⁹ However, SFN dose-dependently inhibited the activities of CYP1A1 and 2B1/2 in rat hepatocytes.²⁹ In other reports, SFN also reduced CYP3A4 activity by decreasing CYP3A4 transcript level in rat hepatocytes.³⁰ It has also been shown that SFN is a potent competitive inhibitor of CYP2E1 with a K_i of 37.0 ± 4.5 mM in microsomes from livers of acetone-treated rats, and SFN is able to inhibit the genotoxicity of *N*-nitrosodimethylamine.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.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, where a strong inverse relationship exists between the tissue levels of these enzymes and susceptibility to chemical carcinogenesis.³³ For example, the Phase II enzyme of GSTs, typically leading to carcinogen detoxification, plays a direct role in the carcinogenic process. Human deficiencies in GSTs expression have been associated with increased cancer risk, such as bladder, oral, and lung cancers.³⁴ Conversely, overexpression of GST-P1 protected human prostate cells from the cytotoxic and DNA damaging action of the prostate carcinogen.³⁵ Most work has focused on Phase II enzyme induction via antioxidant response element (ARE)-driven

gene expression.³⁶ ARE-driven targets include NQO1, HO-1, UGT, and gamma-glutamylcysteine synthetase.³⁷

The modulation of Phase II enzyme activity and gene expression by SFN has been confirmed in a series of cell lines, of which the most commonly used are liver hepatoma cell lines HepG2 and Hepa1c1c7. Some studies indicate that NQO1 and GST activities were increased in the fore stomach, duodenum, and bladder of rats treated with SFN (40 μ M/kg/day), while a higher dose of SFN (200–1,000 μ M/kg/day) increased NQO1 and GST activities in the liver, colon, and pancreas of rats.^{38,39} The effect of SFN on Phase II enzyme regulation has also been studied generally in prostate cancer where SFN was shown to significantly induce the expression and activity of Phase II enzyme in the human prostate cell lines LNCaP, MDA PCa 2a, MDA PCa 2b, PC-3, and TSU-Pr1 treated with 0.1–15 mM of SFN.⁴⁰

Keap1-ARE signaling pathway is an important modifier of susceptibility to electrophilic and oxidative stresses, factors central to the processes of chemical carcinogenesis and other chronic degenerative diseases.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;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.^{43,44} SFN can react with the thiol groups of Keap1 to form thionoacyl adducts, which enables Nrf2 to escape Keap1-dependent ubiquitination and degradation, leading to nuclear localization of Nrf2.^{45,46} The nuclear Nrf2 binds to ARE and activates Phase II detoxifying and antioxidant genes.⁴¹ 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.⁴⁷ The expression and activity of HO-1 by SFN regulation has been shown to be Nrf2 dependent in mouse peritoneal macrophages.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.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-7.⁵⁰ 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 UGT1A1.⁵¹

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.⁴⁵ Constitutive activation of NF- κ B 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.⁵²

NF- κ B 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- κ B expression compared to control cells, suggesting that NF- κ B is an important molecular target of SFN.⁵³

SFN can reduce the DNA-binding activity of NF- κ B directly by indirectly interacting with the thiol groups leading to dithiocarbamate formation and directly uniting with essential cysteine residues of NF- κ B 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- κ B DNA-binding activity.⁵⁴ These findings further confirm that thiol reactivity and redox modulation are important in the regulation of NF- κ B-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.⁵⁵

Thus, the inactivation of NF- κ B 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.⁵⁶ 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.⁵⁷ 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.⁵⁸ 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.⁵⁹ 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.^{60–62} 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.^{61,62} 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.⁶⁰ 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.⁶³

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-9.⁶³ Furthermore, decreased mitochondrial

potential has been detected in prostate and bladder cancer cells in response to SFN.^{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 μ M) for 24 hours can cause a significant increase in the cytosolic protein expression levels of AIF.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.^{67,68} 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- κ B, P13K/AKT, and MEK/ERK pathways in prostate tumor tissues.⁶⁹ However, the activation of the downstream effector caspase-3 has been well documented for SFN in many pathways.^{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.⁷² Furthermore, the expression of the IAP family members c-IAP1 and c-IAP2 was also downregulated with SFN treatment in human glioblastoma cells.⁶⁶

The induction of cell cycle arrest of SFN

The progression of the cell cycle through all four phases, G₁, S, G₂, and M, is adjusted by CDKs and cyclins.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 G₀/G₁, S, and G₂/M phases upon treatment with SFN has been reported in breast,⁷⁴ bladder,⁶⁵ colon,⁷⁵ and prostate cancer cells.^{76,77} Arrest at the G₀/G₁ 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,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-3.⁷⁹ Another study demonstrated that G₂/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.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.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 G₂/M cell cycle arrest and apoptosis, and not due to differences in SFN metabolism or differences in Phase II enzyme induction.⁷⁶

Substantial evidence indicates that the concentration of SFN is responsible for these different effects. G₂/M arrest was observed with treatment of SFN at a dose of 20 μ M, whereas concentrations of 100 μ M induced the accumulation of cells in the sub-G₁ phase, cell death, and dissipation of mitochondrial membrane potential in human colon adenocarcinoma cell Caco-2.⁸¹ SFN at 10 μ M can reduce cell viability and induce G₂/M phase arrest in prostate cancer DU145 cells.⁸² Another G₂/M phase arrest of SFN was observed in ovarian cancer PA-1 cells with 12.5 μ M SFN.⁸⁰ Other evidence also indicates that the growth inhibitory effect of SFN is highly time dependent.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 G₂/M cell cycle arrest and cytostatic growth inhibition even at high concentrations.⁵⁹ After a minimum continuous exposure time of 12 hours, SFN could irreversibly induce cell cycle arrest in the G₂/M phase and the cells appeared to subsequently undergo apoptosis.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.⁸⁵ 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.⁵ 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.⁷⁷ 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.⁷⁴

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.⁸⁶ 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.⁸⁷ 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).⁸⁷ 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.^{88–90} 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.⁹¹

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.

References

1. Anfossi S, Fu X, Nagvekar R, Calin GA. MicroRNAs, Regulatory messengers inside and outside cancer cells. *Adv Exp Med Biol.* 2018; 1056:87–108.
2. Cheung KL, Kong A-N. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *AAPS J.* 2010; 12(1):87–97.
3. Zhou Y, Yang G, Tian H, et al. Sulforaphane metabolites cause apoptosis via microtubule disruption in cancer. *Endocr Relat Cancer.* 2018;25(3):255–268.
4. Linsalata M, Orlando A, Russo F. Pharmacological and dietary agents for colorectal cancer chemoprevention: effects on polyamine metabolism (review). *Int J Oncol.* 2014;45(5):1802–1812.
5. W Watson G, M Beaver L, E Williams D, H Dashwood R, Ho E. Phytochemicals from cruciferous vegetables, epigenetics, and prostate cancer prevention. *AAPS J.* 2013;15(4):951–961.
6. Ramirez CN, Li W, Zhang C, et al. In vitro-in vivo dose response of ursolic acid, sulforaphane, PEITC, and curcumin in cancer prevention. *AAPS J.* 2018;20(1):19.
7. Mori N, Shimazu T, Sasazuki S, et al. Cruciferous vegetable intake is inversely associated with lung cancer risk among current nonsmoking men in the Japan Public Health Center (JPHC) Study. *J Nutr.* 2017;147(5): 841–849.
8. Qj W, Yang Y, Wang J, Han LH, Xiang YB. Cruciferous vegetable consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Cancer Sci.* 2013;104(8):1067–1073.
9. Shwter AN, Abdullah NA, Alshawsh MA, et al. Chemoprevention of colonic aberrant crypt foci by *Gynura procumbens* in rats. *J Ethnopharmacol.* 2014;151(3):1194–1201.
10. Bessler H, Djaldetti M. Broccoli and human health: immunomodulatory effect of sulforaphane in a model of colon cancer. *Int J Food Sci Nutr.* 2018;20(1):1–8.
11. Juengel E, Euler S, Maxeiner S, et al. Sulforaphane as an adjunctive to everolimus counteracts everolimus resistance in renal cancer cell lines. *Phytomedicine.* 2017;27:1–7.
12. Xu C, Shen G, Yuan X, et al. 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.* 2006;27(3):437–445.
13. Royston K, Udayakumar N, Lewis K, Tollefsbol T. A novel combination of withaferin A and sulforaphane inhibits epigenetic machinery, cellular viability and induces apoptosis of breast cancer cells. *Int J Mol Sci.* 2017;18(5):1092.
14. Sivapalan T, Melchini A, Saha S, et al. Bioavailability of glucoraphanin and sulforaphane from high-glucoraphanin broccoli. *Mol Nutr Food Res.* Epub 2017 Dec 21.

15. Yang L, Palliyaguru DL, Kensler TW. Frugal chemoprevention: targeting Nrf2 with foods rich in sulforaphane. *Semin Oncol*. 2016;43(1):146–153.
16. Hanausek M, Walaszek Z, Slaga TJ. Detoxifying cancer causing agents to prevent cancer. *Integr Cancer Ther*. 2003;2(2):139–144.
17. Karin M. Nuclear factor- κ B in cancer development and progression. *Nature*. 2006;441(7092):431–436.
18. Pereira L, Silva P, Duarte M, et al. Targeting colorectal cancer proliferation, stemness and metastatic potential using Brassicaceae extracts enriched in isothiocyanates: a 3D cell model-based study. *Nutrients*. 2017;9(4):368.
19. Frydoonfar HR, McGrath DR, Spigelman AD. Sulforaphane inhibits growth of a colon cancer cell line. *Colorectal Dis*. 2004;6(1):28–31.
20. Hu R, Hebbar V, Kim BR, et al. In vivo pharmacokinetics and regulation of gene expression profiles by isothiocyanate sulforaphane in the rat. *J Pharmacol Exp Ther*. 2004;310(1):263–271.
21. Atwell LL, Zhang Z, Mori M, et al. Sulforaphane bioavailability and chemopreventive activity in women scheduled for breast biopsy. *Cancer Prev Res (Phila)*. 2015;8(12):1184–1191.
22. Franklin SJ, Dickinson SE, Karlage KL, Bowden GT, Myrdal PB. Stability of sulforaphane for topical formulation. *Drug Dev Ind Pharm*. 2014;40(4):494–502.
23. Myzak MC, Tong P, Dashwood WM, Dashwood RH, Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med (Maywood)*. 2007;232(2):227–234.
24. Bergantin E, Quarta C, Nanni C, et al. Sulforaphane induces apoptosis in rhabdomyosarcoma and restores TRAIL-sensitivity in the aggressive alveolar subtype leading to tumor elimination in mice. *Cancer Biol Ther*. 2014;15(9):1219–1225.
25. Tomczyk J, Olejnik A. Sulforaphane – a possible agent in prevention and therapy of cancer. *Postepy Hig Med Dosw (Online)*. 2010;64:590–603.
26. Saha K, Hornyak TJ, Eckert RL. Epigenetic cancer prevention mechanisms in skin cancer. *AAPS J*. 2013;15(4):1064–1071.
27. Meunier B, de Visser SP, Shaik S. Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes. *Chem Rev*. 2004;104(9):3947–3980.
28. Perocco P, Bronzetti G, Canistro D, et al. Glucoraphanin, the bioprecursor of the widely extolled chemopreventive agent sulforaphane found in broccoli, induces Phase-I xenobiotic metabolizing enzymes and increases free radical generation in rat liver. *Mutat Res*. 2006;595(1–2):125–136.
29. Lnenickova K, Dymakova A, Szotakova B. Sulforaphane alters beta-naphthoflavone-induced changes in activity and expression of drug-metabolizing enzymes in rat hepatocytes. *Molecules*. 2017;22(11):E1983.
30. Gross-Steinmeyer K, Eaton DL. Dietary modulation of the biotransformation and genotoxicity of aflatoxin B1. *Toxicology*. 2012;299(2–3):69–79.
31. Barcelo S, Gardiner JM, Gescher A, Chipman JK. CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane. *Carcinogenesis*. 1996;17(2):277–282.
32. Singletary K, Macdonald C. Inhibition of benzo[a]pyrene- and 1,6-dinitropyrene-DNA adduct formation in human mammary epithelial cells by dibenzoylmethane and sulforaphane. *Cancer Lett*. 2000;155(1):47–54.
33. Kwak MK, Egner PA, Dolan PM, et al. Role of Phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat Res*. 2001;480–481:305–315.
34. Landi S. Mammalian class theta GST and differential susceptibility to carcinogens: a review. *Mutat Res*. 2000;463(3):247–283.
35. Nelson CP, Kidd LC, Sauvageot J, et al. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity and DNA adduct formation in human prostate by glutathione S-transferase P1. *Cancer Res*. 2001;61(1):103–109.
36. Anwar-Mohamed A, Elshenawy OH, Soshilov AA, et al. Methylated pentavalent arsenic metabolites are bifunctional inducers, as they induce cytochrome P450 1A1 and NAD(P)H:quinone oxidoreductase through AhR- and Nrf2-dependent mechanisms. *Free Radic Biol Med*. 2014;67:171–187.
37. Liu L, Wu W, Li J, et al. Two sesquiterpene aminoquinones protect against oxidative injury in HaCaT keratinocytes via activation of AMPK α /ERK-Nrf2/ARE/HO-1 signaling. *Biomed Pharmacother*. 2018;100:417–425.
38. Munday R, Munday CM. 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*. 2004;52(7):1867–1871.
39. Matusheski NV, Jeffery EH. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J Agric Food Chem*. 2001;49(12):5743–5749.
40. Brooks JD, Paton VG, Vidanes G. Potent induction of Phase 2 enzymes in human prostate cells by sulforaphane. *Cancer Epidemiol Biomarkers Prev*. 2001;10(9):949–954.
41. Xing H-Y, Cai Y-Q, Wang X-F, et al. The cytoprotective effect of hyperoside against oxidative stress is mediated by the Nrf2-ARE signaling pathway through GSK-3 β inactivation. *PLoS One*. 2015;10(12):e0145183.
42. Hu C, Eggler AL, Mesecar AD, van Breemen RB. Modification of Keap1 cysteine residues by sulforaphane. *Chem Res Toxicol*. 2011;24(4):515–521. 2011/04/18.
43. Qin S, Hou D-X. Multiple regulations of Keap1/Nrf2 system by dietary phytochemicals. *Mol Nutr Food Res*. 2016;60(8):1731–1755.
44. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. *Med Res Rev*. 2012;32(4):687–726.
45. Shimizu T, Inoue K-Ichi, Hachiya H, Shibuya N, Aoki T, Kubota K. Accumulation of phosphorylated p62 is associated with NF-E2-related factor 2 activation in hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci*. 2016;23(8):467–471.
46. Furukawa M, Xiong Y. BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. *Mol Cell Biol*. 2005;25(1):162–171.
47. Shan Y, Wang X, Wang W, He C, Bao Y. p38 MAPK plays a distinct role in sulforaphane-induced up-regulation of ARE-dependent enzymes and down-regulation of COX-2 in human bladder cancer cells. *Oncol Rep*. 2010;23(4):1133–1138.
48. Lin W, Wu RT, Wu T, Khor T-O, Wang H, Kong A-N. Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. *Biochem Pharmacol*. 2008;76(8):967–973.
49. Lee YJ, Jeong HY, Kim YB, et al. Reactive oxygen species and PI3K/Akt signaling play key roles in the induction of Nrf2-driven heme oxygenase-1 expression in sulforaphane-treated human mesothelioma MSTO-211H cells. *Food Chem Toxicol*. 2012;50(2):116–123.
50. Wu J, Williams D, Walter GA, Thompson WE, Sidell N. Estrogen increases Nrf2 activity through activation of the PI3K pathway in MCF-7 breast cancer cells. *Exp Cell Res*. 2014;328(2):351–360.
51. Zy S, Zhang C, Lee JH, et al. Requirement and epigenetics reprogramming of Nrf2 in suppression of tumor promoter TPA-induced mouse skin cell transformation by sulforaphane. *Cancer Prev Res (Phila)*. 2014;7(3):319–329.
52. Kolberg M, Pedersen S, Bastani NE, Carlsen H, Blomhoff R, Paur I. Tomato paste alters NF- κ B and cancer-related mRNA expression in prostate cancer cells, xenografts, and xenograft microenvironment. *Nutr Cancer*. 2015;67(2):305–315.
53. Karmakar S, Weinberg MS, Banik NL, Patel SJ, Ray SK. Activation of multiple molecular mechanisms for apoptosis in human malignant glioblastoma T98G and U87MG cells treated with sulforaphane. *Neuroscience*. 2006;141(3):1265–1280.
54. Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhäuser C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem*. 2001;276(34):32008–32015.
55. Thejass P, Kuttan G. 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- γ . *Immunopharmacol Immunotoxicol*. 2006;28(3):443–457.

56. Pérez-Garijo A, Steller H. Spreading the word: non-autonomous effects of apoptosis during development, regeneration and disease. *Development*. 2015;142(19):3253–3262.
57. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cell Mol Life Sci*. 2007; 64(9):1105–1127.
58. Lenzi M, Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer. *Cancer Treat Res*. 2014;159:207–223.
59. Parnaud G, Li P, Cassar G, et al. Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. *Nutr Cancer*. 2004;48(2):198–206.
60. Choi YH. ROS-mediated activation of AMPK plays a critical role in sulforaphane-induced apoptosis and mitotic arrest in AGS human gastric cancer cells. *Gen Physiol Biophys*. 2018;37(02):129–140.
61. Lan H, Yuan H, Lin C. Sulforaphane induces p53-deficient SW480 cell apoptosis via the ROS-MAPK signaling pathway. *Mol Med Rep*. 2017;16(5):7796–7804.
62. Park S, Kim G, Bae S-J, Yoo Y, Choi Y. Induction of apoptosis by isothiocyanate sulforaphane in human cervical carcinoma HeLa and hepatocarcinoma HepG2 cells through activation of caspase-3. *Oncol Rep*. 2007;18(1):181–187.
63. Lee C-SU, Cho H-JI, Jeong YUN-J, et al. Isothiocyanates inhibit the invasion and migration of C6 glioma cells by blocking FAK/JNK-mediated MMP-9 expression. *Oncol Rep*. 2015;34(6):2901–2908.
64. Negrette-Guzmán M, Huerta-Yepez S, Vega MI, et al. Sulforaphane induces differential modulation of mitochondrial biogenesis and dynamics in normal cells and tumor cells. *Food Chem Toxicol*. 2017; 100:90–102.
65. Islam SS, Mokhtari RB, Akbari P, Hatina J, Yeager H, Farhat WA. Simultaneous targeting of bladder tumor growth, survival, and epithelial-to-mesenchymal transition with a novel therapeutic combination of acetazolamide (AZ) and sulforaphane (SFN). *Target Oncol*. 2016;11(2):209–227.
66. Karmakar S, Roy Choudhury S, L. Banik N, K. Ray S. Activation of multiple molecular mechanisms for increasing apoptosis in human glioblastoma T98G xenograft. *J Cancer Sci Ther*. 2010;02(04):107–113.
67. Jin C-Y, Molagoda IMN, Karunarathne WAHM, et al. TRAIL attenuates sulforaphane-mediated Nrf2 and sustains ROS generation, leading to apoptosis of TRAIL-resistant human bladder cancer cells. *Toxicol Appl Pharmacol*. 2018;352:132–141.
68. Żuryń A, Litwiniec A, Safiejko-Mroccka B, et al. The effect of sulforaphane on the cell cycle, apoptosis and expression of cyclin D1 and p21 in the A549 non-small cell lung cancer cell line. *Int J Oncol*. 2016; 48(6):2521–2533.
69. Shankar S, Ganapathy S, Srivastava RK. Sulforaphane enhances the therapeutic potential of TRAIL in prostate cancer orthotopic model through regulation of apoptosis, metastasis, and angiogenesis. *Clin Cancer Res*. 2008;14(21):6855–6866.
70. Park HS, Han MH, Kim G-Y, et al. Sulforaphane induces reactive oxygen species-mediated mitotic arrest and subsequent apoptosis in human bladder cancer 5637 cells. *Food Chem Toxicol*. 2014;64: 157–165.
71. Wang N, Wang W, Liu C, Jin J, Shao B, Shen L. Inhibition of growth and induction of apoptosis in A549 cells by compounds from oxheart cabbage extract. *J Sci Food Agric*. 2016;96(11):3813–3820.
72. Choi S, Lew KL, Xiao H, et al. D,L-Sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. *Carcinogenesis*. 2007;28(1):151–162.
73. Gubern A, Joaquin M, Marqués M, et al. The N-terminal phosphorylation of RB by p38 bypasses its inactivation by CDKs and prevents proliferation in cancer cells. *Mol Cell*. 2016;64(1):25–36.
74. Royston KJ, Paul B, Nozell S, Rajbhandari R, Tollefsbol TO. Withaferin A and sulforaphane regulate breast cancer cell cycle progression through epigenetic mechanisms. *Exp Cell Res*. 2018;368(1):67–74.
75. Psurski M, Janczewski Łukasz, Świtalska M, et al. Novel phosphonate analogs of sulforaphane: synthesis, invitro and invivo anticancer activity. *Eur J Med Chem*. 2017;132:63–80.
76. Clarke JD, Hsu A, Yu Z, Dashwood RH, Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. *Mol Nutr Food Res*. 2011;55(7):999–1009.
77. Hsu A, Wong CP, Yu Z, Williams DE, Dashwood RH, Ho E. Promoter de-methylation of cyclin D2 by sulforaphane in prostate cancer cells. *Clin Epigenetics*. 2011;3(1):3:3.
78. Chatterjee S, Rhee Y-H, Ahn J-C. Sulforaphane–carboplatin combination synergistically enhances apoptosis by disruption of mitochondrial membrane potential and cell cycle arrest in human non-small cell lung carcinoma. *J Med Food*. 2016;19(9):860–869.
79. Bryant CS, Kumar S, Chamala S, et al. Sulforaphane induces cell cycle arrest by protecting RB–E2F-1 complex in epithelial ovarian cancer cells. *Mol Cancer*. 2010;9(1):47.
80. Chang C-C, Hung C-M, Yang Y-R, Lee M-J, Hsu Y-C. Sulforaphane induced cell cycle arrest in the G2/M phase via the blockade of cyclin B1/CDC2 in human ovarian cancer cells. *J Ovarian Res*. 2013; 6(1):41.
81. Jakubíková J, Sedláč J, Mithen R, Bao Y. 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*. 2005;69(11):1543–1552.
82. Yang SH, Chien CM, Chang LS, Lin SR. Involvement of c-jun N-terminal kinase in G2/M arrest and caspase-mediated apoptosis induced by cardiotoxin III (*Naja naja atra*) in K562 leukemia cells. *Toxicol*. 2007; 49(7):966–974.
83. Peng X, Zhou YAN, Tian HUA, et al. Sulforaphane inhibits invasion by phosphorylating ERK1/2 to regulate E-cadherin and CD44v6 in human prostate cancer DU145 cells. *Oncol Rep*. 2015;34(3):1565–1572.
84. Pappa G, Bartsch H, Gerhäuser C. Biphasic modulation of cell proliferation by sulforaphane at physiologically relevant exposure times in a human colon cancer cell line. *Mol Nutr Food Res*. 2007;51(8): 977–984.
85. Pandey MK, Gupta SC, Nabavizadeh A, Aggarwal BB. Regulation of cell signaling pathways by dietary agents for cancer prevention and treatment. *Semin Cancer Biol*. 2017;46:158–181.
86. Myzak MC, Dashwood WM, Ormer GA, Ho E, Dashwood RH. Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apc-minus mice. *Faseb J*. 2006;20(3):506–508.
87. Zhang C, Su Z-Y, Khor TO, Shu L, Kong A-NT. Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. *Biochem Pharmacol*. 2013;85(9):1398–1404.
88. Shapiro TA, Fahey JW, Dinkova-Kostova AT, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical Phase I study. *Nutr Cancer*. 2006;55(1):53–62.
89. Cornblatt BS, Ye L, Dinkova-Kostova AT, et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis*. 2007;28(7):1485–1490.
90. Alunkal JJ, Slottke R, Schwartzman J, et al. A Phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer. *Invest New Drugs*. 2015;33(2):480–489.
91. Lozanovski VJ, Houben P, Hinz U, Hackert T, Herr I, Schemmer P. Pilot study evaluating broccoli sprouts in advanced pancreatic cancer (POUDER trial) – study protocol for a randomized controlled trial. *Trials*. 2014;15(1):204.

Drug Design, Development and Therapy**Dovepress****Publish your work in this journal**

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are the features of the journal, which

has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/drug-design-development-and-therapy-journal>