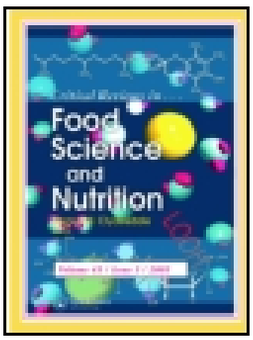

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REVIEW

Metabolism, absorption, and anti-cancer effects of sulforaphane: an update

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ABSTRACT

Cancer is one of the most devastating diseases, and recently, a variety of natural compounds with preventive effects on cancer developments have been reported. Sulforaphane (SFN) is a potent anti-cancer isothiocyanate originating from *Brassica oleracea* (broccoli). SFN, mainly metabolized via mercapturic acid pathway, has high bioavailability and absorption. The present reviews mainly discussed the metabolism and absorption of SFN and newly discovered mechanistic understanding recent years for SFN's anti-cancer effects including promoting autophagy, inducing epigenetic modifications, suppressing glycolysis and fat metabolism. Moreover, its inhibitory effects on cancer stem cells and synergetic effects with other anti-cancer agents are also reviewed along with the clinical trials in this realm.

KEYWORDS

Cancer; cancer stem cells; metabolism; novel mechanisms

Introduction

As population ages, cancer incidence is climbing rapidly, and the newly diagnosed cases will skyrocket to over 22 million annually by 2035 (Kelly et al. 2015). To address, novel drugs and strategies are needed for cancer inhibition and therapy. However, chemotherapeutics induce severe side effects, and dietary compounds are used as alternatives for cancer prevention and suppression (Bhatti and Salama 2018; Gu, Mao, and Du 2020).

Sulforaphane (SFN) is an isothiocyanate derived from *Brassicaceae* vegetables such as *Brassica oleracea* (broccoli). It is released when natural glucoraphanin is hydrolyzed by exogenous myrosinase enzymes or gut microflora (Angelino and Jeffery 2014; Lai, Miller, and Jeffery 2010; Fahey et al. 2012). Since the re-discovery of SFN in 1992 and the potency of broccoli sprouts found in Johns Hopkins University (Fahey, Zhang, and Talalay 1997; Zhang et al. 1992), SFN have acquired wide attentions as a green and effective chemo protector. It is a natural agent against a great number of chronic diseases, such as intestinal inflammation caused by *Helicobacter pylori* (Yanaka et al. 2009), type 2 diabetes (Axelsson et al. 2017) and autism spectrum disorder (Singh et al. 2014). Moreover, SFN is a potent and green anti-cancer agent fighting against various cancers (breast cancer, skin cancer, oral carcinoma, and so on; Dinkova-Kostova et al. 2006; Bauman et al. 2015; Fahey, Zhang, and Talalay 1997). Over the past decades, a large body of researches focusing on anti-cancer effects of SFN have been reported, and these effects and underlying mechanisms have also been successively summarized by Lenzi, Fimognari, and Hrelia (2014), Atwell et al. (2015a), and Russo et al. (2018). These possible mechanisms including modulation of phase I/II enzymes and DNA repair enzymes, cyclins, induction of antioxidant defenses, cell cycle arrest, classic apoptosis signaling, inhibition of vascular endothelial growth factor, metal matrix proteins (1, 2, and 9), inflammation signaling, and regulation of transcription factor nuclear factor E2-related factor 2 (Nrf2) (Russo et al. 2018; Lenzi, Fimognari, and Hrelia 2014; Atwell et al. 2015a). Yagishita and colleagues have comprehensively summarized SFN clinical studies including formulation, bioavailability and efficacy of glucoraphanin and/or SFN applied in these preclinical and clinical trials, as well as choices of doses and route of administration (Yagishita et al. 2019).

During the last several years, mechanistic understanding for SFN's anti-cancer effects continues to accumulate, and newly discovered mechanisms include modulation of autophagy, induction of epigenetic modifications, suppression of glycolysis and fat metabolism, and synergism with other anti-cancer agents. Furthermore, SFN is a promising compound to inhibit cancer stem cells (CSCs) (Xie et al. 2019; Liu, Peng, et al. 2017; Moura et al. 2016; Ge et al. 2019), the root cause for tumor-initiation, regrowth and invasiveness. Meanwhile, like other phytochemicals, SFN is amphipathic and instable, both metabolism and absorption of the agent is crucial for its outstanding anti-cancer effects. Thus, in the present review, we discussed the updated anti-cancer information of SFN reported recent years and the metabolism and absorption of agents, and also discussed some clinical trials in this realm.

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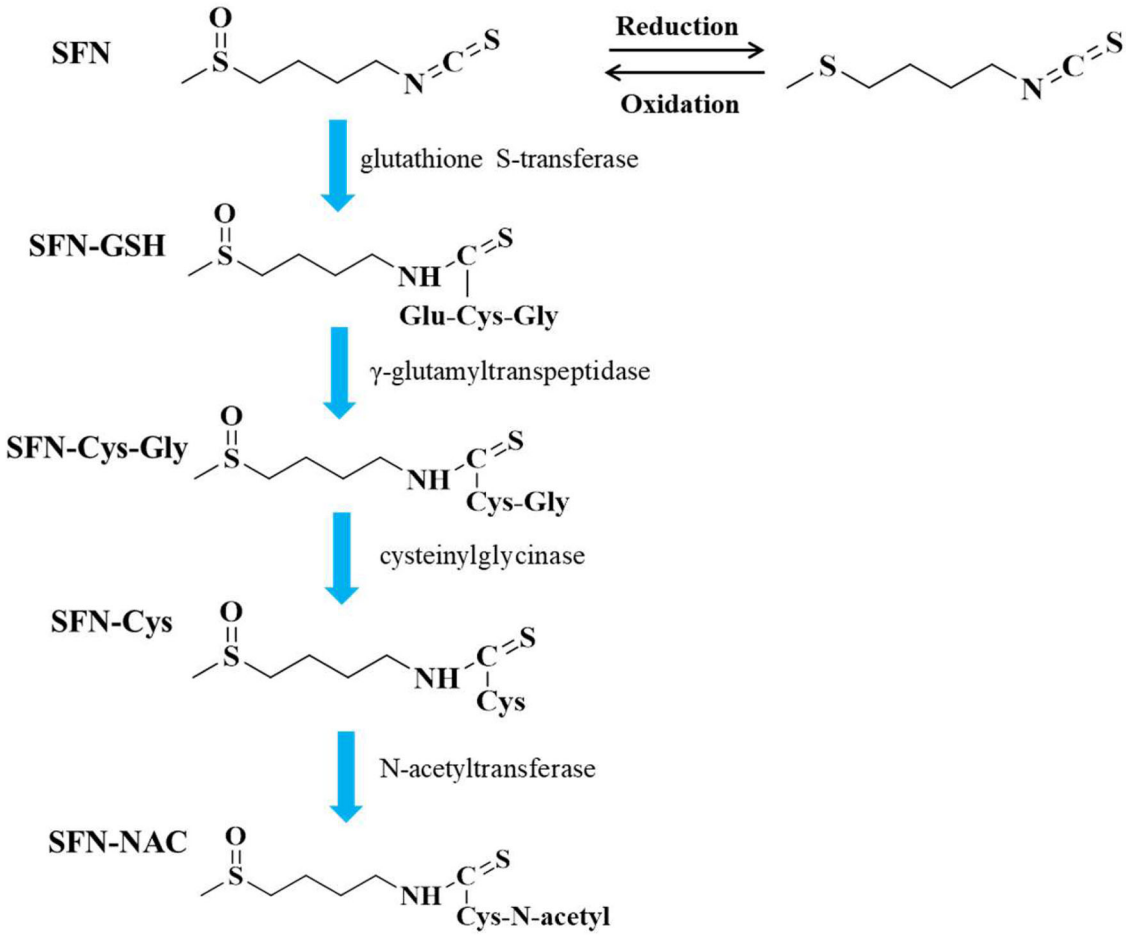

Figure 1. Metabolic scheme of sulforaphane (SFN) via mercapturic acid pathway and biotransformation between SFN and erucin. SFN-GSH, sulforaphane-glutathione; SFN-NAC, SFN-N-acetylcysteine.

Metabolism and absorption of sulforaphane

SFN, like other isothiocyanates, shares the –N=C-S group with an electrophilic carbon atom (Kostov et al. 2017). SFN is metabolized via mercapturic acid pathway (Figure 1), which is triggered by the spontaneous reaction between electrophilic –N=C-S group and glutathione in vivo. Initially, under the catalysis of glutathione S-transferase (GST), electrophilic –N=C-S group of SFN reacts with electronegative sulfhydryl groups of glutathione (GSH) spontaneously, forming SFN-glutathione (SFN-GSH) (Zhang et al. 1995; Mi, Di Pasqua, and Chung 2011). Then, SFN–GSH is gradually catalyzed by γ-glutamyltranspeptidase, cysteinylglycinase and N-acetyltransferase, forming SFN-Cys-Gly, SFN-Cys and SFN-N-acetylcysteine (SFN–NAC). These metabolites come into the circulation and are excreted in the urine finally (Zhang et al. 1995). The parent SFN and metabolites reach the highest levels at 1–3 h after administration and are cleared within 24 h in human plasma (Ahmed et al. 2006; Egner et al. 2008). The main metabolite of SFN is SFN-NAC (Thomas-Ahner et al. 2012). A similar metabolic change of SFN is observed in health dogs except that the main metabolite is SFN-GSH in plasma (Curran et al. 2018). Additionally, SFN can also reversibly convert into erucin (an oxidized form of SFN) by reduction reaction in mice and HCT 116 lung cancer cells (Thomas-Ahner et al. 2012; Platz et al. 2015).

Generally, the bioavailability and absorption of SFN is high. Once ingested orally, SFN is metabolized and eliminated quickly in vivo, the half-life of which is about 2.067 ± 0.255 h and 70–90% of SFN are excreted in the form of its metabolites (Fahey et al. 2017). Similarly, both of human and animal studies confirmed that most SFN (74% or more) was recovered as its metabolites in the urine within 24 h post-ingestion (Cramer and Jeffery 2011), confirming the high bioavailability and absorption of SFN in humans. In animals, the concentration of SFN and its metabolites could reach micromolar level in the plasma of mouse gavaged with 295 μmol/kg of SFN (Thomas-Ahner et al. 2012). In cultured cells, SFN accumulates inside human and animal cells quickly, and reach the highest concentration (millimolar range) within 0.5–3 h (Zhang 2001). Once going into cells, SFN quickly interacts with GSH to form SFN–GSH, leading to its intracellular accumulation (Zhang 2001; Mi, Di Pasqua, and Chung 2011). This property favors SFN accumulation in cancer cells due to their high GSH content, promoting the anti-cancer effect of SFN.

In clinical research, considering the instability of SFN, formulations rich in glucoraphanin (a stable precursor of SFN), such as beverages made of fresh or lyophilized broccoli sprout extracts, are usually used, and the SFN bioavailability of these formulations are also studied (Fahey et al. 2012; Egner et al. 2011). Myrosinase can convert

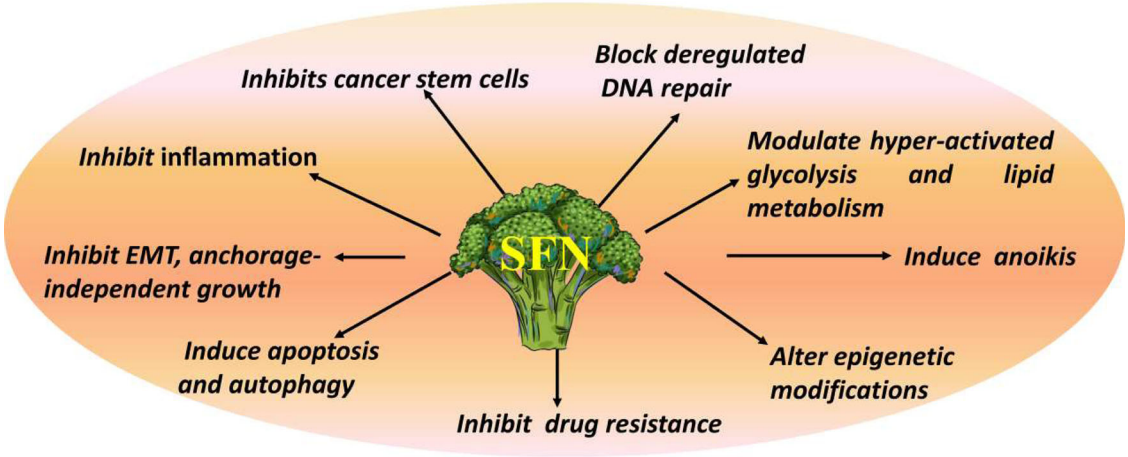

Figure 2. Mechanisms contributing to anti-cancer effects of sulforaphane (SFN).

glucoraphanin to SFN, thus the amount and activity of the enzyme is closely related to the SFN bioavailability of the formulations. A cross-over clinical trial shows that SFN bioavailability in people solely consuming glucoraphanin is much less than that of people directly consuming SFN, and interindividual variability in glucoraphanin group is higher than that of SFN group (Egner et al. 2011). These differences might due to the inadequate myrosinase merely derived from gut microflora and different gut microbiome composition and performance of each subject (Egner et al. 2011). Meanwhile, glucoraphanin displays a more stable steady-state amount of SFN in vivo which is in favor of exerting the lasting chemo preventive effects of SFN (Egner et al. 2011). Similarly, in another clinical study, formulations composed of both glucoraphanin and active enzyme myrosinase show greater SFN bioavailability (3–4 times) than that of formula without active myrosinase (Fahey et al. 2015). In addition, Fahey and colleagues confirms that omeprazole (a proton pump inhibitor) or enteric coating myrosinase can improve the SFN bioavailability of formulations (broccoli sprout extract and active myrosinase) (Fahey et al. 2019). These might due to the protection of myrosinase from the attacking of acidic environment in stomach. Thus aiming to achieve the greater SFN bioavailability and better bioactive effects, proper formulations should be used in clinical. Egner et al proposed that mixture of SFN and glucoraphanin would be the optimal choice, because the mixture could achieve peak concentrations for activation of some targets (such as Nrf2) and sustained concentrations for the inhibition of signalings (such as apopotosis) involved in healthy function of SFN (Egner et al. 2011).

Sulforaphane suppresses carcinogenesis via various mechanisms sulforaphane induces epigenetic alteration

Carcinogenesis is closely associated with epigenetic dysregulation including global DNA hypomethylation but and hypermethylation in tumor-suppressor genes. Epigenetic dysregulation is partly responsible for the initiation and progression of carcinomas (Bennett and Licht 2018). And ameliorating epigenetic dysregulation is a promising choice for the treatment of cancer. SFN is a natural histone deacetylase inhibitor, which is partly responsible for its anti-cancer effect (Figure 2 and Table 1). SFN inhibits breast cancer development by suppressing histone deacetylases 5 (HDAC5) via downregulating upstream transcription factor 1, a factor controlling transcription of HDAC5 (Cao et al. 2018). Similarly, HDAC 5 and 11 are also inhibited by SFN in hepatoma carcinoma cells accompanied by the increased methylation level of genes involved in cell cycle (E2F3), cell proliferation (THAP1) and anti-apoptosis (ANKHD1), which partly accounts for the cell arrest, apoptosis and DNA damage induced by SFN (Patrick et al. 2020). Interestingly, low concentration of SFN (5–10 μM) induces global DNA hypomethylation and inhibits HDAC 3, 4, 6, 7, 8, 9, 10 in MDA-MB-231 cells, which might contribute to the anti-cancer effect of SFN (Lewinska et al. 2017). Additionally, human telomerase reverse transcriptase (hTERT) is overexpressed in most of cancers in favor of infinite proliferation. SFN (1.25–5 μM) attenuates the expression of hTERT via downregulating HDAC 1, inhibiting the proliferation of colon cancer cells (Martin, Kala, and Tollefsbol 2018).

MicroRNAs (miRNAs), modulating genes expression at the transcriptional level, are novel biomarkers for diagnosis and prognosis of cancers. SFN can prevent malignancy by downregulating onco-miRNAs and pro-metastatic miRNAs and upregulating anti-cancer miRNAs. Upon treatment by SFN, three onco-miRNAs (miR-23b, miR-92b, and miR-381) are decreased and their downstream target TGFB2 is upregulated accordingly in breast cancer cells, which in-turn increase the expression of CDKN1A, a cell cycle blocker (Lewinska et al. 2017). MiR-616-5p is a pro-metastatic miRNA highly expressed in lung cancer cells (Lewinska et al. 2017). SFN inhibits miR-616-5p transcription by decreasing H3K9Ac and upregulating H3K9me3. Subsequently, GSK3β/β-catenin signaling, a downstream pathway of miR-616-5p, was inactivated, leading to the suppressing of epithelial-mesenchymal transition (EMT) (Wang et al. 2017). Similarly, SFN also blocks EMT via increasing miR-200c expression in bladder cancer cells (Huang et al.

Table 1. Anti-cancer effects and potential targets of sulforaphane.

Major mechanisms	Cells/animals	Dose	Effect	Potential targets	References
Modulation of epigenics	MDA-MB-231 cells	25 μM; 50 mg/kg	Inhibit tumor growth	[a]↓USF1 ↓HDAC5 ↓LSD1 [b]↑LSD1 deubiquitinase USP28	Cao et al. (2018)
	HepG2	8, 16, and 32 μM	Trigger apoptosis and DAN damage; Induce cell cycle arrest and cell viability; Induce mitotic spindle abnormalities;	↓HDAC5 and HDAC11 ↑DNA methylation	Patrick et al. (2020)
	HCT 116 and RKO cells	10 μM	Inhibit cell viability and morphological changes; Induce apoptosis	↓miR-21 ↓HDAC1 and hTERT ↓HDAC and telomerase activity	Martin, Kala, and Tollefsbol (2018)
	MCF-7, MDA-MB-231and SK-BR-3 cells	5, 10, 20μM	Induce cell cycle arrest Induce apoptosis Induce energy stress and autophagy Induce nitro-oxidative stress Promote senescence Induce global DNA hypomethylation	↑p21 and p27 ↓p-AKt ↑p-AMPK/ AMPK ↓DNMT1 and DNMT3B ↓m6A RNA methylation ↓miR-23b, -92b, -381, and -382	Lewinska et al. (2017)
	H1299, 95C, and 95D cells; Male BALB/c nude mice	5 μM	Inhibit cell proliferation; Inhibit migration and invasion; Inhibit EMT;	↓miR-616-5p ↑E-cadherin; ↓N-cadherin and Vimentin; ↓β-catenin ↓miR-616-5p/GSK3β/β-catenin signaling; ↓H3K9Ac; ↑H3K9me3	Wang et al. (2017)
	T24 and UMUC-3 cells	10 μM	Inhibit EMT; Inhibit invasion;	↑E-cadherin; ↓Vimentin, ZEB1; ↑miR -200c; ↑miRA-200c/ ZEB1 axis	Huang et al. (2018)
	A549	2.5, 5.0 μM	–	↑miR-9-3; ↓CpG methylation; ↑H3K4me1 enrichment; ↓DNMT3a, HDAC1, HDAC3, HDAC6 and CDH1 ↓DNMT activity	Gao et al. (2018)
	BxPC-3 cells	10 μM	Inhibit cell viability, self-renewal; Inhibit tumor growth	↑miR135b-5p; ↑RASAL2	Yin et al. (2019)
	AGS and MKN45cells	31.25, 62.5, 125, and 250 μg/ml	Inhibit cell viability; Induce apoptosis;	↑CDX1, ↓CDX2; ↑miR-9, -326	Kiani et al. (2018)
	H1299, 95C, and 95D cells; Male BALB/c nude mice	5 μM	Inhibit cell proliferation; Inhibit migration and invasion; Inhibit EMT;	↓miR-616-5p ↑E-cadherin; ↓N-cadherin and Vimentin; ↓β-catenin ↓miR-616-5p/GSK3β/β-catenin signaling; ↓H3K9Ac; ↑H3K9me3	Wang et al. (2017)
	LNCaP and PC-3 cells	15 μM	–	↓LINC01116	Beaver et al. (2017)
	SK-1 and A549 cells	15μM			Geng et al. (2017)

Inducing apoptosis and autophagy			Induce apoptosis; Induce mitochondrial fusion; Increase 26S proteasome activity;	↑p-ERK1/2; ↑Cleaved caspase 3; ↑Bax; ↓Bim; ↑26S proteasome	
	A549 and SK-1 cells	SFN-Cys 10, 20, 30 μM	Inhibited cell proliferation; Induce apoptosis and alteration of cell morphology;	↑p-ERK1/2 ↑maspin ↑Bax, cleaved caspase-3; ↓pro-caspase-3, Bcl-2, α-tubulin;	Lin et al. (2017)
	CL1-0 and CL1-5 cells	10 -40 μM	Inhibit viability and clonogenic ability; Induce ROS production; Induce cell-cycle arrest in S-phase Induce apoptosis Inhibit tumor growth	↑γH2AX ↑LC3-II ↑cleaved caspase-9, -7	Wang et al. (2019)
	MDA-MB-231, BT549 and MDA-MB-468 cells; BALB/c nu/nu athymic nude mice	10 μM; 50 mg/kg	Induce autophagosome formation; Induce autophagy; Inhibit tumor growth	↑Beclin1 ↑LC3-II ↓P62 ↓HDAC6 ↑membrane PTEN	Yang et al. (2018)
	A549 and SK-1 cells	SFN-Cys 10, 15 μM SFN-NAC 15 μM	Inhibit migration, invasion and wound healing ability; redistribution of α-tubulin; Induce autophagosomes formation; Inhibit autolysosome formation;	↑p-ERK1/2; ↓Claudin-5; ↑Claudin-7; ↓α-tubulin; ↑LC3 II/LC3 I;	Zheng et al. (2019)
Modulation of inflammation	MDA-MB-231 MCF-7 SK-BR-3 4T1.2	1 mg SFN/mouse	Inhibit osteoclast defferention Inhibit osteolytic bone resorption	↓RUNX2 ↓CTSK ↓IL8 ↓NF-κB	Pore et al. (2020)
	HT-29 and RKO cells	1.25–5μM	–	↓inflammatory cytokine (IL-2, IFNc, and IL-1ra)	Bessler and Djaldetti (2018)
Modulation of glycolysis and fat synthesis	RT112 and RT4 cells	20 μM	Inhibit cell proliferation; Inhibit glycolytic metabolism;	↓Ki67 ↓HIF-1α; ↓Nuclear translocation of HIF-1α	Xia et al. (2019)
	LNCaP and 22Rv1 cells	10 μM	Decrease extracellular acidification rate; Decrease levels of lactate in plasma and prostate tumor	↓hexokinase II ↓pyruvate kinase M2 ↓lactate dehydrogenase A	Singh et al. (2019)
	LNCaP and 22Rv1 cells; TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice	5, 10 μM; 6 μM/mouse	Inhibit fatty acid synthesis; Decrease levels of total FFA, total phospholipids,acetyl-CoA and ATP; Prevent β-oxidation of fatty acids Decrease neutral lipid droplets	↓acetyl-CoA carboxylase 1; ↓fatty acid synthase; ↓carnitine palmitoyltransferase 1A; ↓β-oxidation dehydrogenases; ↓SREBP1;	Singh et al. (2018)
Other mechanisms	A549 and H1299 cells	0–3 μM 50 mg/kg	Inhibit EMT; Inhibit wound healing, migrating and invasive ability; Induce cell morphological change Inhibit tumor growth	↑E-cadherin, ZO-1 ↓N-cadherin, Snail1 ↓MMP2 ↑p-ERK5 ↑ERK5 ↓p-c-Jun and ↓p-c-Fos	Chen et al. (2019)
	A549 and CL1-5 cells	10–40 μM	Induce anoikis Inhibit anchorage-independent growth	↓β-catenin ↑p21 ↓p-FAK, p-Akt,	Tsai, Tsai, and Wu (2019)
	HT-29 cells	10, 30, 50 μM			

(*continued*)

Table 1. Continued.

Major mechanisms	Cells/animals	Dose	Effect	Potential targets	References
			Inhibit cell viability and wound-healing ability; Induce apoptosis	↓mRNA level of COX-2, mPGES-1, VEGF, CXCR4; ↓mRNA level of MMP-2, -9 and HIF-1; ↑Caspases-3; ↓COX-2/mPGES-1 axis;	Tafakh et al. (2019)
	HCT116 and SW480 cells; polyposis in rat colon (Pirc) model	tetrazole-containing heterocyclic analogs of SFN 15 μM	Induce DNA damage, cell cycle arrest; Induce apoptosis; Trigger the alteration of HAT/HDAC activities	↓HDAC 3 ↑pH2AX levels ↓C-terminal binding protein, interacting protein; ↓P300/CBP- associated factor; ↓lysine acetyltransferase 2A;	Okonkwo et al. (2018)

a ↓Represents downregulation.
b ↑Represents upregulation.
Abbreviations: AMPK, adenosine monophosphate activated protein kinase; Cox-2, cyclooxygenase-2; CXCR4, CXC chemokine receptor 4; DNMT; ERK, extracellular signal regulated kinase; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase 3β; HDAC, histone deacetylase; IL6, Interleukin 6; LSD1, lysine-specific demethylase 1; hTERT, human telomerase reverse transcriptase; HDAC, histone deacetylase; HIF-1α, hypoxia-inducible factor; micro-RNAs, miR; MMP, matrix metalloproteinase; mPGES-1, membrane associated prostaglandin E; m^6A, N^6-methyladenosine; NF-κB, nuclear factor-κB; SERBP1, sterol regulatory element binding proteins1c; RUNX2, runt-related transcription factor 2; USF1, upstream transcription factor 1;VEGF, vascular endothelial growth factor.

2018). MiR-9-3 is an anti-cancer miRNAs downregulated in tumor cells, and SFN restores miR-9-3 by inducing demethylation and acetylation of its promoter, which may account for the anti-cancer effect of the drug (Gao et al. 2018). MiR135b-5p is also an anti-cancer miRNA newly found in pancreatic ductal adenocarcinoma, and SFN effectively blocks self-renewal and tumor growth of pancreatic ductal adenocarcinoma via upregulation of miR135b-5p and activating its target gene RASAL2 (Yin et al. 2019). In gastric cancer, both miR-9 and miR-326, two tumor suppressors, are downregulated by SFN, which is related to the decreased cell viability and increased cell apoptosis (Kiani et al. 2018). Apart from directly inhibiting cancers, SFN also attenuates drug resistance via regulating miRNAs. Gap junctions are hemichannels in the plasma membrane that facilitate drugs exchanging among cells. However, they are commonly deficient in cancer cells, leading to the chemoresistance. SFN enhances connexin 43 expression and gap junction formation via decreasing the level of miR30a-3p (a blocker of connexin 43), leading to the attenuation of drug-resistance in pancreatic cancer. Thus drugs co-treated with SFN may display more potent chemotherapy effect (Georgikou et al. 2020).

Like miRNA, long noncoding RNAs (lncRNAs) are also targets of SFN in cancer inhibition. LINC01116 is overexpressed in prostate cancers, which is downregulated in prostate cancer cells following treatment by SFN (15 μM) (Beaver et al. 2017).

Sulforaphane triggers apoptosis and autophagy signaling

Apoptosis is a programmed cell death and accurately regulated by various signal pathways under normal conditions. However, apoptosis-resistance is commonly observed in cancers, leading to infinite growth of malignant tumor. Thus restoring apoptosis is a vital strategy to fight against cancer. SFN induces apoptosis via multiple pathways (Figure 2 and Table 1), which are discussed as follows. SFN downregulates bim (a member of Bcl-2 family) via activation of extracellular signal regulated kinase 1/2 (ERK1/2) in lung cancer cells (Geng et al. 2017). Additionally, SFN-Cys, an analog of SFN, triggers apoptosis via upregulation of maspin, a tumor suppressor, in lung cancer cells, which is blocked when co-treatment with PD98059 (an ERK1/2 inhibitor), indicating that ERK1/2 activation mediates upregulation of maspin and the consequent apoptosis (Lin et al. 2017). Elevated expression of epidermal growth factor receptor, which stimulates ERK1/2 signaling, abolished apoptosis elicited by SFN in lung cancer. (Wang et al. 2019)

Autophagy plays dual roles (pro-cancer and anti-cancer) in the progression of cancers depending on the contexts (Li, He, and Ma 2020). In triple negative breast cancer, SFN inhibits cell viability and tumor growth in vivo via the activation of autophagy, which was confirmed by the formation of autophagosome and upregulation of autophagic markers (LC-II and Beclin 1; Yang et al. 2018). On the other hand, SFN-NAC can inhibit autophagy via stabilization of

microtubule, leading to the attenuation of migration and invasion in lung cancer cells (Zheng et al. 2019). Thus, the roles of autophagy in mediating the anti-cancer effects of SFN require further investigation.

Sulforaphane inhibits inflammatory signaling

Cancers induce local inflammation, which promotes metastasis. SFN was able to inhibit osteoclast differentiation triggered by breast cancer partly due to the inhibition of nuclear factor-κB (NF-κB) signaling (Figure 2 and Table 1), a classical inflammatory pathway, which is mediated by runt-related transcription factor 2 (RUNX2) blockage (Pore et al. 2020). When co-cultured with colon cancer cells, SFN suppresses the release of pro-inflammation factors such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) by human peripheral blood mononuclear cells (Bessler and Djaldetti 2018).

Sulforaphane modulates glycolysis and lipid metabolism

Malignant cells depend on glycolysis to provide energy, which produces lactate and form an acidic tumor microenvironment which favors tumor metastasis and chemoresistance (Li et al. 2016). Thus inhibition of glycolysis generates anti-cancer effects. SFN inhibits propagation of bladder cancer cells by inhibition of glycolysis (Figure 2 and Table 1), which is mediated by downregulation of hypoxia-inducible factor 1 and its nuclear-translocation (Xia et al. 2019). Similarly, SFN decreases glycolysis of prostate cancer in vitro and in vivo (Singh et al. 2019). However, no significant effects on glycolysis were observed for prostate cancer patients consuming SFN-rich broccoli sprout extracts (Singh et al. 2019).

Dysregulation of lipid metabolism is another metabolic hallmark of malignant cells such as increased de novo fatty acid synthesis, enhanced lipid absorption and steatolysis, which provide energy for the rapidly proliferating malignant cells (Liu, Peng, et al. 2017). Thus restoration of deregulated lipid metabolism is also a strategy for cancer treatment. In prostate cancer cells, SFN inhibits fatty acid synthesis and decreases the amount of intracellular lipid droplets by suppressing the expression of acetyl-CoA carboxylase 1 and fatty acid synthase, both of which are responsible for fatty acid synthesis (Singh et al. 2018). Additionally, SFN also prevents fatty acids absorption by decreasing carnitine palmitoyl-transferase 1A which regulates β-oxidation of fatty acids (Singh et al. 2018).

Sulforaphane inhibits cancer stem cells

Cancer stem cells (CSCs) are a small group of cancer cells existing in malignant tumors, which are characterized by high drug-resistance, potent metastatic and invasive ability and infinite self-renewal. Mounting evidences confirm that CSCs are the root for initiation and relapse of malignant tumors (Najafi, Farhood, and Mortezaee 2019; Chang et al. 2015). SFN can inhibit CSCs via various mechanisms, such as targeting self-renewal signaling, activating apoptosis and autophagy pathways and altering miRNAs (Table 2).

Targeting self-renewal pathways

SFN is capable of inhibiting CSCs self-renewal via blockage of Wnt/β-catenin, Hedgehog and Notch signaling. SFN reduces aldehyde dehydrogenase 1 $^{+}$ ($ALDH^{+}$) CSCs by 65% to 80% and mammospheres formation, suggesting its effectiveness in eliminating breast CSCs. Such inhibition effect is due to attenuation of β-catenin-mediated transcription (Li et al. 2010). Consistently, SFN-loaded nanoparticles suppress Wnt/β-catenin signaling, thereby reducing breast CSCs ($ESA^{+}CD44^{+}CD24^{-}$) (Huang et al. 2016). In addition, SFN represses the stemness of pancreatic CSCs in vitro and in vivo as indicated by inhibition of tumorospheres formation and expression of proteins contributing to stemness (Oct4 and Nanog), which is partly due to the blockage of sonic hedgehog signaling (Rodova et al. 2012, Li et al. 2013). Similarly, SFN inhibits gastric CSCs partly via suppressing sonic hedgehog signaling (Ge et al. 2019). Additionally, high expression of Np63α stimulates Notch signaling, leading to the acquisition of CSC-like properties in human bronchial epithelial cells; SFN counteracts these effects by downregulating IL-6 and consequently blunting Notch signaling and CSC-like properties (Xie et al. 2019). Similarly, SFN can perturb the expression of YAP1 (a transcriptional adaptor protein of Hippo pathway), preventing spheroid forming, invasive, migrating and tumor-initiative capability of CSCs derived from epidermal squamous cell carcinoma (Fisher et al. 2017).

Targeting apoptosis signaling

SFN also inhibits CSCs via induction of apoptosis. In pancreatic cancer, SFN (20 μM) suppresses Bcl-2 (an anti-apoptosis protein) and activates caspases-dependent apoptosis signaling (Rodova et al. 2012). Consistently, SFN also prevents gastric CSCs partly via inducing apoptosis, which is confirmed by increased expression of Bax and caspases 3, 8, 9 and downregulation of Bcl-2 (Ge et al. 2019).

Targeting micro-RNAs

Modulation of micro-RNAs contributes to the anti-CSCs effect of SFN. Exosomal miR-140 secretion is lower in MCF10 basal-like ductal carcinoma stem cells accompanied by highly expression of miR-21 and miR-29. SFN increases exosomal miR-140 expression and decreases both miR-21 and miR-29, which reduce ALDH1 levels and decrease mammosphere formation (Li et al. 2014a). In addition, SFN restores miR-140, inhibits tumorigenicity of MCF10DCIS stem-like cells in immunodeficient nude female mice and decreases percentage of CSCs ($CD44^{high}/CD24^{low}$) in MDA-MB-231 cells (Li et al. 2014b). MiR-200c inhibits tumorigenicity of head and neck squamous CSCs by negatively regulating Bmi1, a factor maintaining cell stemness (Lo et al. 2011). SFN downregulates Bmi1 via increasing the expression of miR-200c, thereby preventing expression of

Table 2. Inhibitory effects of Sulforaphane on CSCs.

Major mechanism	Type of cancer	Sulforaphane Concentration	Anti-CSCs effects	Potential molecular targets	References
Inhibiting self-renewal signaling	breast cancer	1–5μM 50 mg/kg	Inhibit mammosphere formation; Reduce ALDH$^+$ cells; Induce apoptosis; Inhibit tumor growth in vivo	[b]↑Caspase-3 activity [a]↓β-catenin ↓β-catenin transcriptional ↓nuclear β-catenin ↓cyclin D1 ↓p-GSK3β (Ser9)	Li et al. (2010)
	pancreatic Cancer	5–20 μM	Inhibit spheroid formation; Induce apoptosis	↓Nanog, Oct-4 ↓PDGFRa, Cyclin D1 ↓Bcl-2 ↑cleaved Caspase-3 ↓Smo, Gli1 and Gli2	Rodova et al. (2012)
	pancreatic cancer	20 mg/kg/d	Induce apoptosis; Inhibit tumor growth and proliferation of tumor cells	↓Smo, Gli 1, and Gli 2 ↓Nanog and Oct-4 ↓VEGF and PDGFRa ↓Zeb-1 ↑E-Cadherin ↓Bcl-2 and XIAP ↓Smo, Gli 1, and Gli 2 ↓Sonic hedgehog–GLI signaling	Li et al. (2013)
	gastric cancer	1, 5, 10 μM	Inhibit number and size of tumor sphere, cells proliferation, Induce apoptosis	↓CD133, CD44 ↓Oct-4, Nanog ↑Cyclin D1, PCNA ↓Bcl-2 ↑Bax, Caspase 8, Cleaved caspase 3, 9, ↓Shh, Smo, Gli1, and Gli2,	Ge et al. (2019)
	lung cancer	5-15μM; 25 or 50 mg/kg for 21d	Decrease number and size of tumor sphere; Inhibit tumor growth in vivo	↓CD133, ALDH1A1 ↓Oct4, Nanog ↓NICD, Hes1, IL-6, ΔNp63α ↓IL-6/ΔNp63α/Notch axis	Xie et al. (2019)
	epidermal squamous cell carcinoma	20 μM	Inhibit invasion, migration, spheroid formation Inhibit tumor growth	↓YAP1, ΔNp63α ↑p- YAP1 ↓YAP/ΔNp63α signaling	Fisher et al. (2017)
Modulation of miRNAs	breast cancer	10–20μM	Inhibit mammosphere formation; Reduce progenitor colony formation; Reduce ALDH1 expression;	↑miR-140 ↓miR-21 and miR-29	Li et al. (2014a)
	breast cancer	–	Inhibit tumor growth Decrease stem-like cell frequency	↑miR-140 ↓ALDH1/Sox9	Li et al. (2014b)
	oral squamous cell carcinomas	20, 40 μM 50 mg/kg	Inhibit cell proliferation; Inhibit migration, invasion, colony formation; Inhibit self-renewal ability Inhibit tumor growth Decrease proportion of ALDH + Cells/ CD44+ Cells	↑miR-200c ↓Bmi1	Liu, Peng, et al. (2017)
	Non-small cell lung cancer	5, 10μM; 4mg/kg/3d	Inhibit tumor sphere formation; Decrease population of CD133+ cells and ALDH + cells; Inhibit tumor growth in vivo; Improve chemotherapeutic effect	↓c-Myc, Cyclin E1, EZH2 ↓β-catenin ↓miR-214	Li et al. (2017)
	Lung cancer	1, 5, 15 μM	Inhibit tumor sphere formation; Induce sphere-forming cells apoptosis	↓CSCs marker (CD133, CD44, ALDH1) ↓c-Myc, Cyclin D1, PCNA ↓Bcl-2, Bax, Caspase 8, Cleaved Caspase 9	Zhu et al. (2017)

			↑Cleaved Caspase 3 ↓p-GSK3β, β-catenin ↑GSK3β ↓miR-19/GSK3β/β-catenin axis	
Other mechanism	Prostate cancer	5, 10, 20 μM;	↓c-Myc ↓DLL4 and ITGA6	Moura et al. (2016)
			Inhibit colony formation; Decrease ALDH1 activity and Proportion of CD49f + cells; Inhibit 1st and 2nd formation of tumor sphere	

[a] ↓Represents downregulation.
[b] ↑Represents upregulation.
Abbreviations: ALDH, aldehyde dehydrogenase; Bcl-2, B-cell lymphoma 2; CD, cluster of differentiation; GSK3β, glycogen synthase kinase 3β; PDGFRa, platelet-derived growth factor receptor a; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

CSCs markers, migrating and invasive ability of oral squamous CSCs in vitro and tumor-initiative ability in vivo (Liu, Peng, et al. 2017). c-Myc is a pro-cancer factor and involved in maintaining CSCs (Kim et al. 2010); SFN inhibits the expression of c-Myc and increases the level of miR-214 which directly binds to the coding region of c-Myc and also downregulates β-catenin signaling, thereby inhibiting lung CSCs properties in vitro and in vivo (Li et al. 2017). In another study, SFN effectively attenuates the trait of lung CSCs by suppressing the level of miR-19, which further targets GSK3β to deactivate Wnt/β-catenin signaling (Zhu et al. 2017).

Other mechanisms

Epithelial-mesenchymal transition (EMT) plays critical roles in gaining invasiveness and metastasis of cancer. Recently, SFN diminishes EMT in lung cancer cells and xenografted H1299 lung tumor which is mediated by the activation of ERK 5 (Chen et al. 2019). Apart from EMT, resistance to detachment-triggered apoptosis (such as anoikis) and anchorage-independent growth are closely related to the metastatic potential of cancer cells. SFN is confirmed to impede metastasis of lung cancer cells by stimulating anoikis and inhibiting anchorage-independent growth via inactivation of focal adhesion kinase (FAK) and protein kinase B (Akt) signaling in a p53-dependent manner (Tsai, Tsai, and Wu 2019). Prostaglandin E2 (PGE2) is overexpressed in colon cancer cells, which fuels the metastatic, anti-apoptotic and proliferative ability. SFN downregulates the expression of PGE2 by inhibiting cyclooxygenase-2 and microsomal PGE synthase-1, both of which are responsible for the synthesis of PGE2 from arachidonic acid, thereby leading to apoptosis, poor viability and wound-healing ability of HT-29 colon cancer cells (Tafakh et al. 2019). In addition, excessive activation of DNA repair signaling is responsible for the drug-resistance of cancer cells. In rat colon polyposis, SFN analogs are capable of stimulating apoptosis and cell arrest by inducing DNA repair signaling (Okonkwo et al. 2018). SFN diminishes the prostate CSCs-like properties (level of aldehyde dehydrogenase 1, proportion of $CD49f^{+}$ cells and tumorigenecity) by decreasing the expression of c-Myc (Moura et al. 2016).

Sulforaphane combined with other chemotherapeutics improves anti-cancer effects

Due to its effectiveness in inhibiting cancer growth, SNF is also used together with other drugs or compounds (Table 3).

5-fluorouracil is a first line chemotherapy drug for triple-negative breast cancer, the most refractory cancer among the breast carcinoma. Recently, it was found that sequentially treated MDA-MB-231 with SFN and 5-fluorouracil achieved synergistic effects (combination index <1) and consequently induced both autophagic cell death and senescence (Milczarek et al. 2018). In another study, SFN combined with cisplatin achieved synergetic effects on the anti-

Table 3. Synergistic effects of sulforaphane in combination with chemotherapy drugs on cancer cells and CSCs.

Type of cancer	Cells/animals	Combined treatment	Synergistic anti-CSCs effects	Potential molecular targets	References
Breast cancer	MDA-MB-231 cells	SFN +5-fluorouracil	Increase number of autophagic vacuoles; Induce mitotic slippage; Increase population of cells inG2/M phase; Induce premature senescence;	[b]↑LC3-II [a]↓Cyclin B ↓senescence marker p21 protein	Milczarek et al. (2018)
Ovarian cancer	A2780 and OVCAR cells	SFN (2.5–10 μM) + cisplatin	Suppress cell proliferation; Enhance induction of apoptosis	↓Bcl-2 ↓Cyclin-D1 ↓c-Myc ↑P53 ↑Cleaved Caspase-3	Kan, Wang, and Sun (2018)
Breast cancer	MDA-MB-231 and MCF7 cells	SFN (5 μM) +withaferin A (1 μM)	Promote cells death; Induce cell cycle arrest; Increase global methylation	↓Cyclin D1 mRNA ↓CDK4 mRNA ↓pRB ↑E2F mRNA ↑p21 ↑H3K4Me3	Royston et al. (2018)
Breast cancer	MCF-7 cells	SFN (20 μM) + $1,25(OH)_2D_3$(100 nM)	Decrease nuclear HDACI/II enzymeactivity Inhibit colonyformation; Decrease cell viability; Induce apotosis	–	Hossain, Liu, and Wood (2020)
Colon cancer	HCT116 and SW480 cells	sulforaphane (5, 10 μM)+L.pentosus S-PT84 (10 μg/mL)	increased the induction of apoptosis; Decrease mitochondria membrane potential;	↑TNFα ↑TNFR1 ↓XIAP ↑Bcl-2	Yasuda, Horinaka, and Sakai (2019)
Colon cancer	HT-29 Cells	sulforaphane (30 μM)+ R8-PNA-a15b (8 μM)	Enhance induction of apoptosis; Enhance inhibition of cell proliferation	↑mRNA of caspase-3, p53, and BAK1; ↓miR-15b-5p	Jessica et al. (2020)
Prostate cancer	Prostate CSCs	SFN (10 μM) + TRAIL (5 ng/mL)	Enhance the inhibition of colony and tumor sphere formation; Inhibit ALDH1 activity	↓SOX2 and Nanog ↓CD133, CD44 ↓Ki67 ↓NF-κB singaling ↓CXCR4, Notch 1, Jagged1	Labsch et al. (2014)
Gastric cancer	Gastric CSCs	SFN (10 μM) + cisplatin (2 μM)	Inhibit colony formation Decrease side population and ratios of CD44+/EpCAM + cells	↓IL-6R, STAT3, and p-STAT3 ↓cyclinD1 and c-Myc ↑miR-124	Wang et al. (2016)
Pancreatic cancer	Pancreatic CSCs	SFN (10 μM)+ Sorafenib (20 μM)	Inhibit Colony and spheroid formation Decrease ALDH + Cells ratio; Inhibit proliferation , angiogenesis, EMT; Inhibit tumor growth;	↓caspase-3/7, caspase-8, 9 ↓cIAP, XIAP, and cFLIP ↓HIF-1α ↓Vimentin, Twist2, Zeb-1 ↓Ki67 ↓NF-κB singaling	Rausch et al. (2010)
Chronic leukemia Cancer	Chronic Leukemia CSCs	SFN (30 μM)+imatinib (0.1, 1 μM)	Increase apoptosis; Enhance intracellular ROS; Decrease intracellular GSH	↑caspase 3, cleaved PARP, Bax ↓Bcl-2 ↓BCR-ABL, p-CRKL,β-catenin ↓ MDR-1 BCR-ABL signaling	Lin et al. (2012)

[a] ↓Represents downregulation.
[b] ↑Represents upregulation.
Abbreviations: Bcl-2, B-cell lymphoma 2; CDK, cyclin-dependent kinase; CXCR4, CXC chemokine receptor 4; HIF-1α, hypoxia-inducible factor; IL-6R, Interleukin 6 receptors; MDR, multidrug resistance proteins; NF-κB, nuclear factor-κB; STAT3, signal transducer and activator of transcription 3; TNFR, tumor necrosis factor receptor; XIAP, xlinked inhibitor of apoptosis protein.

Table 4. Clinical studies focusing on anti-cancer effects of sulforaphane[a].

NCT number	Title	Condition	Number of subjects	Intervention	Phase	Status
NCT03232138	Clinical Trial of Lung Cancer Chemoprevention With Sulforaphane in Former Smokers	Lung Cancer	-[b]	Daily oral dose 120 μmol SFN (four tablets 2 times per day)	II	Recruiting
NCT03182959	Broccoli Sprout Extract in Preventing Recurrence in Patients With Tobacco-Related Head and Neck Squamous Cell Cancer	Head and Neck Cancer; Head and Neck Squamous Cell Carcinoma; Tobacco-Related Carcinoma Carcinoma in Situ Dysplasia Hyperplasia Premalignant Lesion	36	First cycle: Daily oral dose 70 μmol SFN (Avmacol®) at evening for 28d; Second cycle: Daily oral dose 140 μmol SFN (Avmacol®) at evening for 28d	Early I	Active, not recruiting
NCT01228084	Sulforaphane in Treating Patients With Recurrent Prostate Cancer	Adenocarcinoma of the Prostate/ Recurrent Prostate Cancer	20	Daily oral dose 200 μmol SFN (four 50 μmol capsules) for 20 weeks	II	Completed
NCT00982319	Study to Evaluate the Effect of Sulforaphane in Broccoli Sprout Extract on Breast Tissue	Breast Cancer	34	100 μmol SFN (dissolved in 150 mL mango juice) once a day for 5 weeks	II	Completed
NCT00843167	Broccoli Sprout Extract in Treating Women Who Have Had a Mammogram and Breast Biopsy	Breast Cancer Precancerous Condition	54	Orally intake broccoli sprout extract 3 times per day for 2–8 weeks	II	Completed
NCT01265953	Chemoprevention of Prostate Cancer, HDAC Inhibition and DNA Methylation (PBroC)	Prostate Cancer Prevention	98	200 μmol of SFN daily (capsules of broccoli sprout extract)	I	Completed
NCT00946309	Effects of Sulforaphane on Normal Prostate Tissue (PHASE)	Prostate Cancer	45	100 μmol SFN every other day for 5 weeks	I	Completed
NCT01437501	Broccoli Sprout Intervention in Qidong, P.R. China	Environmental Carcinogenesis	291	600 μmol glucoraphanin + 40 μmol SFN daily for 84 day (mixed in 100 mL dilute pineapple and lime juice)	II	Completed
NCT02656420	Broccoli Sprout Dose Response: Bioavailability and Effects of Air Pollutants	Environmental Carcinogenesis	170	120 μmol glucoraphanin +8 μmol SFN; 200 μmol glucoraphanin +20 μmol SFN; 600 μmol glucoraphanin +40 μmol SFN orally (mixed with pineapple juice, lime juice and water.)	I	Completed

[a]The informations of the clinical studies is acquire from website (https://clinicaltrials.gov/ct2/home).
[b]Represent the number is still unknown.

proliferation and pro-apoptosis role against ovarian cancer cells (Kan, Wang, and Sun 2018). Apart from chemotherapy drugs, SFN is combined with other dietary compounds to fight against cancers. Withaferin A originating from winter cherry is a DNA methyl transferase suppressor. Treatment of breast cancer cells with both SFN and withaferin A accelerated cell death and cycle arrest by upregulating p21 tumor suppressor (Royston et al. 2018). Additionally, joint treatment with SFN and vitamin D 3 (VD3) achieved potent preventing effects on breast cancer cells by inactivating HDAC I/II activity (Hossain, Liu, and Wood 2020). Similarly, compared with the individual agent, combined use of SFN with multiple phytochemicals (lycopene, quercetin, and curcumin) enhanced the inhibitory effects on colon cancer cells (Langner, Lemieszek, and Rzeski 2019). R8-PNA-a15b is a cell penetrating peptide-nucleic acid and can effectively inhibit the expression of miRNA-15b-5p, a pro-cancer miRNAs. Co-treatment of HT-29 lung cancer cells with SFN and R8-PNA-a15b induced stronger effects on apoptosis and proliferation than that of individual drug (Jessica et al. 2020).

CSCs are commonly more refractory to the conventional chemotherapy agents, and SFN was introduced to potentiate the anti-CSCs effects of these drugs. Gefitinib resistance is often observed in the clinical treatment for lung carcinoma. SFN suppresses gefitinib tolerance of the established gefitinib-resistant PC9 cells, similar to lung CSCs, by suppressing sonic hedgehog pathway (Wang et al. 2018). Similarly, leukemia stem cells display high resistance to imatinib, a first line drug for treating chronic leukemia cancer. However, jointly treatment with SFN and imatinib made leukemia CSCs more sensitive to imatinib and triggered apoptosis of these cells, which was mainly due to the heightened blockage of Wnt/β-catenin signaling (Lin et al. 2012). In addition, chemotherapy drugs often cause side effects, such as stimulating inflammation and excessive DNA toxicity, constraining their application in cancer therapy. Sorafenib was found to activate NF-κB signaling in pancreatic CSCs, leading to relapse of tumor spheroids. Because SFN is an inhibitor of NF-κB signaling, co-treatment with SFN and sorafenib achieve a potent synergetic effect on eradication of pancreatic CSCs (Rausch et al. 2010). Similar synergetic effect was also found in the treatment of prostate CSCs by both SFN and TRAIL, an anti-cancer agent which could also trigger NF-κB signaling (Labsch et al. 2014). Besides, cisplatin, a standard drug for the treatment of gastric cancer, was restrained due to the severe toxicity. When combining cisplatin (2 μM) with SFN(10 μM) to treat gastric cells, SFN enhanced the anti-gastric CSC effects of low dose cisplatin, which was explained by repressing miR-124/IL-6R/STAT3 axis (Wang et al. 2016).

Clinical studies focusing on anti-cancer effects of sulforaphane

Due to the potent anti-cancer effects of SFN found in studies based on cells and animals, some clinical trials have been conducted (Table 4). A phase II trial (NCT number: NCT03232138) aims to reveal whether SFN intervention (120 μmol/d) improve the conditions of former smokers who are at high risk of developing cancer. The drug used in the trail is Avmacol®, it is made from broccoli sprout and seed extract powder and is also experiencing an early phase 1 clinical trial (NCT number: NCT03182959). Now, the trail is still under recruiting status. Another phase II trial (NCT number: NCT01228084) reports that daily intervention by the capsule of broccoli sprout extracts (BSE) (SFN 200 μmol) for 20 weeks cannot significantly decrease prostate-specific antigen (PSA) level in most of patients with recurrent prostate cancer, while it confirms that the supplement formulation is safe (Alumkal et al. 2015). Similarly, a double-blind, randomized controlled trial (NCT number: NCT01265953) shows that administration of BSE capsule (SFN 200 μmol) do not induce marked alteration of HDAC activity or prostate tissue biomarkers except for those of related gene (Zhang et al. 2020). In terms of breast cancer, women positive for breast cancer are supplemented with glucoraphanin in 2- to 8-week double-blinded, randomized controlled trial, the result show that the supplementation is safe but not enough to change the level of tumor biomarkers. Thus more amounts of glucoraphanin and longer intervention might be considered in the future (Atwell et al. 2015b). Similar, in another study (NCT number: NCT00982319), female breast cancer patients are supplemented with BSE (SFN 100 μmol) daily for 5 weeks, the result also confirms the safety of BSE. Apart from evaluating the directly effect of SFN on cancers, researchers from John Hopkins University also test detoxification of SNF on carcinogenic substance (phenanthrene and aflatoxin) (NCT number: NCT01437501 and NCT02656420). The former trial shows that consumption of glucosinolate from broccoli sprout might be in favor of reducing the toxicity of phenanthrene and aflatoxin, because the concentration of dithiocarbamates (metabolites of glucosinolate) in urine is inversely associated with that of the two carcinogenic substance (Kensler et al. 2005). The latter study shows that intervention by the formulation (600 μmol glucoraphanin + 40 μmol SFN) daily for 12 weeks can significantly reduce the toxicity of benzene and acrolein, both of which are airborne pollutants (Egner et al. 2014).

Conclusions

SFN has acquired a wide range of attentions in cancer prevention and therapy. Herein, the metabolism and absorption of SFN, its newly discovered anti-cancer mechanisms, and its synergetic antic-cancer effects with other drugs are reviewed. And some clinical trials focusing on evaluating the anti-cancer effects of SFN are also discussed. SFN is metabolized via mercapturic acid pathway, and displays high bioavailability. SFN inhibits cancers via various novel mechanisms such as modulation of autophagy, altering epigenetics, suppressing glycolysis and fat metabolism and drug-resistance, and preventing CSCs via regulating self-renewal signaling, miRNAs and apoptosis pathways. However, most available studies in vitro treated cancer cells with SFN for 48 h or more, which is more than the circulation time (less than 24 h) of the drug and its metabolites,

thus sufficient administration and repeated intake of SFN should be obeyed for clinical use. Secondly, although there are studies focusing on the synergetic effect of SFN and natural compounds against differentiated cancer cells, no similar reports were found in the inhibition of CSCs, thus treatment by two or more green agents with SFN should be further investigated, which might achieve outstanding effect via the multi-targeted treatments. Finally, although SFN displays potent anti-cancer effects in vitro studies, the effect displayed in clinical studies is weak or negligible. This might be due to the insufficient amount of SFN released by the formulation, most of which is glucoraphanin. Thus factors (myrosinase, pH, and gut microbiome) affect the conversion rate of glucoraphanin to SFN should be considered and optimal formulations should be developed and selected in the future. Overall, SFN is indeed a safe and potent anti-cancer agent by orchestrating various molecular targets and pathways, and more studies need to be conducted to further enhance its efficiency.

Disclosure statement

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