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Cruciferous Vegetables, Bioactive Metabolites, and Microbiome for Breast Cancer Prevention

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Abstract

Breast cancer is a heterogeneous disease with varying subtypes, prognoses, and treatment responses. Cruciferous vegetables have shown promise in reducing breast cancer risk. This review discusses (*a*) the efficacy of sulforaphane (SFN) and indole-3-carbinol (I3C)/3,3′-diindolylmethane (DIM) on breast cancer risk, prognosis, and treatment outcomes in recent human studies through 2024; (*b*) preclinical studies (2018–2024) that evaluate the efficacy and synergism of SFN, DIM, and other phytochemicals with conventional breast cancer treatments as promising combination therapy strategies for validation in future clinical trials; and (*c*) the role of the microbiome in breast cancer and the interaction between interindividual variations in gut microbiome and glucosinolate metabolism that could modify the benefits of cruciferous vegetable consumption and breast cancer treatment efficacy. Integrating cruciferous vegetables and their bioactive compounds in light of an individual's microbiome profile as a complementary approach alongside standard treatments is a promising strategy in breast cancer care.

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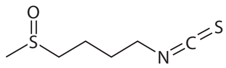
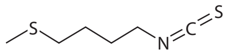
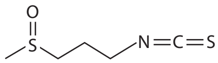
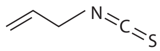
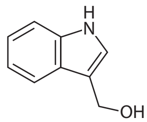
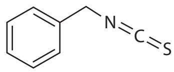
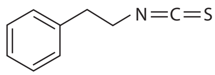
INTRODUCTION

Breast cancer is a significant cause of cancer-related deaths worldwide. In the United States, breast cancer is the second most common cancer in women, with approximately one in eight women (13%) expected to develop breast cancer in their lifetime. In 2024, an estimated 310,720 new cases of invasive breast cancer among women will be diagnosed, representing 32% of all new cancer cases ($n = 972{,}060$) in women (93). Breast cancer is a heterogeneous disease with varying subtypes, prognoses, and sensitivity to therapies. Diet plays an important role in modifying breast cancer risk, with various dietary components, nutrients, and food additives associated with increased or decreased cancer risk (72). Among the potential dietary factors with chemoprevention properties, cruciferous vegetables have shown promise in reducing breast cancer risk and have been the subject of numerous preclinical, clinical, and epidemiological studies.

Cruciferous vegetables are a diverse group of vegetables, characterized by their cross-shaped flower petals, and belong to the Brassicaceae family (45). Cruciferous vegetables are rich sources of glucosinolates (GLSs), a class of sulfur-containing phytochemicals associated with numerous health benefits (45). Upon consumption of cruciferous vegetables, GLSs undergo hydrolysis by plant or bacterial myrosinase (released during damage to the plant through chopping or chewing, and by exposure to intestinal microbiota) to produce various bioactive

Table 1 Structure and major sources of cruciferous vegetables–derived bioactive metabolites

Class	Glucosinolate	Isothiocyanate	Structure	Major sources
Aliphatic	Glucoraphanin	Sulforaphane		Broccoli, Brussels sprouts, cauliflower, kale, kohlrabi
	Glucoerucin	Erucin		Arugula, broccoli
	Glucoiberin	Iberin		Broccoli, cabbage, kale
	Singrin	Allyl-isothiocyanate		Broccoli, Brussels sprouts, cabbage, horseradish, kohlrabi, radish
Indole	Glucobrassicin	Indole-3-carbinol		Broccoli sprouts, Brussels sprouts, cabbage, cauliflower
Aromatic	Glucotropaeolin	Benzyl-isothiocyanate		Cabbage, garden cress, Indian cress
	Gluconasturtiin	Phenethyl isothiocyanate		Watercress

metabolites including isothiocyanates (ITCs) and indoles, both of which have been widely studied for their anticancer properties (**Table 1**) (23, 45, 76, 85). ITCs with reported chemopreventive properties include sulforaphane (SFN), allyl isothiocyanate, benzyl isothiocyanate, and 2-phenethyl isothiocyanate. Among these, SFN is the most potent and is derived from the major GLS precursor in broccoli, glucoraphanin (GFN) (7). Among the indoles, indole-3-carbinol (I3C) is the predominant bioactive product derived from the GLS precursor glucobrassicin that is abundant in cabbage and Brussels sprouts. In the stomach, under acidic conditions, I3C is converted into 3,3′-diindolylmethane (DIM), the predominant acid condensation product (7, 41, 114).

Both I3C and DIM have been widely studied for their anticancer properties and effects on estrogen metabolism (1, 118). In preclinical studies using various cancer cell types and tumor models, the anticancer properties of SFN and I3C/DIM are effective in inhibiting multiple stages of cancer development, including tumor initiation, progression, and invasion. The anticancer effects are multifaceted and are mediated through numerous mechanisms and cellular processes, including cell proliferation, cell cycle arrest, DNA repair, autophagy, angiogenesis, inflammation, carcinogen detoxification, and oxidative stress (**Figure 1**) (47, 85). Some of these processes are regulated via epigenetic mechanisms that modulate the expression and activities of histone deacetylase (HDAC), DNA methylation, microRNA, and long noncoding RNA (4). SFN and I3C/DIM can also suppress tumor progression by inhibiting cancer stem cells (CSCs) involved in cancer renewal and differentiation as well as epithelial-mesenchymal transition (EMT), which are involved in tumor migration and invasion (22, 51). In sum, SFN and I3C/DIM contribute to the inhibition of both tumor initiation and tumor progression/metastasis.

Despite extensive investigations of SFN and I3C/DIM's chemopreventive properties in preclinical models, the evidence for the benefits of cruciferous vegetable consumption and the clinical efficacy of interventions using SFN (fresh broccoli sprouts or broccoli sprout extracts

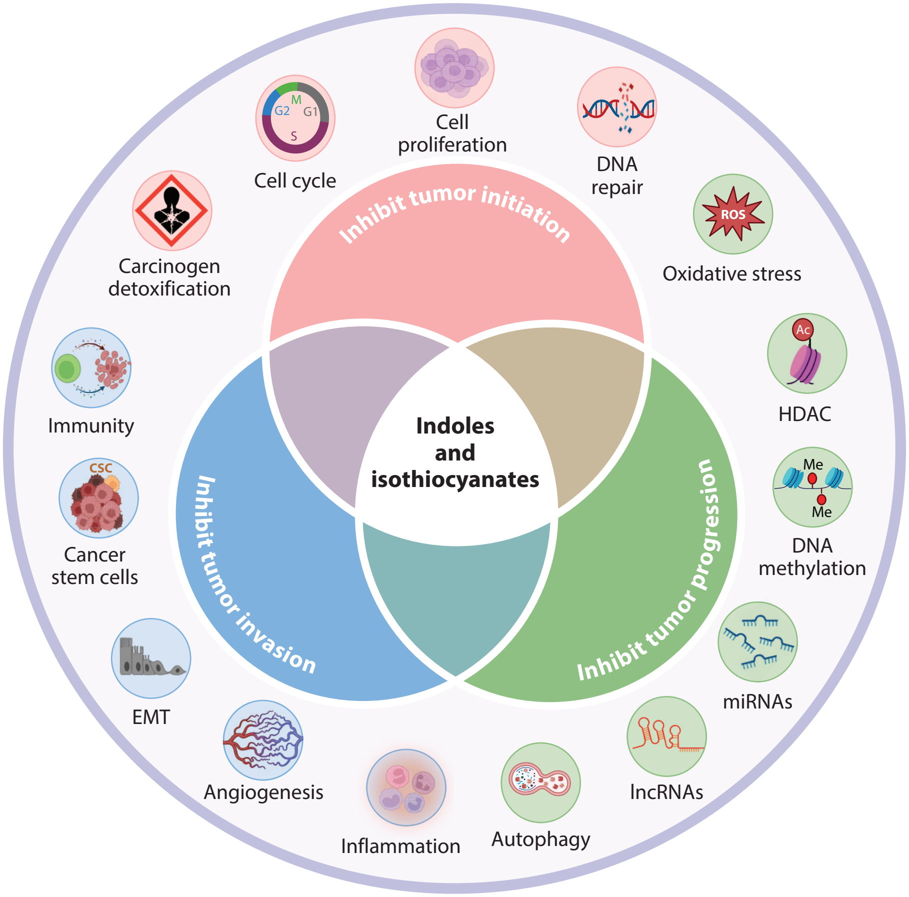


Figure 1

Overview of biological mechanisms of indoles and isothiocyanates (ITCs) in breast cancer prevention and different stages of tumor development: tumor initiation, progression and invasion. The anticancer effects of indoles and ITCs involve multiple mechanisms. The outer ring outlines the diverse molecular and cellular targets, with the placement of each representing their primary effects on different stages of tumor development. Tumor initiation includes processes such as carcinogen detoxification, cell cycle regulation, inhibition of cell proliferation, and enhancement of DNA repair mechanisms. Tumor progression includes processes such as oxidative stress [reactive oxygen species (ROS)] reduction, induction of autophagy, modulation of histone deacetylases (HDACs), DNA methylation, and regulation of microRNAs (miRNAs) and long noncoding RNA (lncRNAs). Tumor invasion includes mechanisms like suppression of epithelial-mesenchymal transition (EMT), inhibition of angiogenesis and cancer stem cells (CSCs), reduction of inflammation, and enhancement of anticancer immunity. These anticancer effects are not limited to a single phase of tumor development, as illustrated by the Venn diagram, which demonstrates an overlapping and interconnection of these anticancer mechanisms. Figure adapted from images created in BioRender; Wong C. 2024. **https://biorender.com/a53v986**.

supplementation) and I3C/DIM (supplements) in breast cancer chemoprevention remains uncertain. The aim of this article is to present a review on the most recent literature on cruciferous vegetables, their derived bioactive metabolites, and their associations with breast cancer. This review summarizes human studies up to 2024, investigating the relationships between cruciferous vegetable intake and breast cancer risk and prognosis, as well as clinical trials examining the effects of cruciferous vegetable–derived compounds on breast cancer outcomes. Furthermore, there is growing interest in exploring the use of SFN and I3C/DIM in combination with standard cancer treatments to improve therapeutic efficacies. This article also discusses recent advances (2018–2024) regarding the chemopreventive efficacy of GLSs, ITCs, and indoles in breast cancer preclinical models, emphasizing the efficacy and synergism of SFN and I3C/DIM when combined with various cancer treatments and other bioactive phytochemicals. Finally, the article explores the potential role of the gut microbiome on various aspects of breast cancer, including its contribution to interindividual variations in GLS metabolism.

EFFICACY OF CRUCIFEROUS VEGETABLES AND THEIR DERIVED COMPOUNDS ON BREAST CANCER RISK, PROGNOSIS, AND INTERVENTION

Cruciferous Vegetables and Breast Cancer Risk and Prognosis (Observational Studies)

Since the 2013 meta-analysis summarizing literature up to 2011, which showed significant inverse associations between cruciferous vegetables and breast cancer (62), six additional studies have investigated cruciferous vegetables and related compound intakes, in association with breast cancer risk, all reporting significant inverse associations (**Table 2**). Among these, three are case-control studies. Zhang et al. (131) first reported that lower intake of cruciferous vegetables, GLS or ITC was associated with higher risk of breast cancer for both pre- and postmenopausal women, and for both ER+ (estrogen receptor positive) and ER– (estrogen receptor negative) breast cancer (1,485 cases and 1,506 controls). Zhang et al. (132) further analyzed the impact of glutathione S-transferase (GST) polymorphisms within a subset of this population (737 breast cancer cases and 756 controls), reporting no significant interactions between cruciferous vegetable, GLS, or ITC intake and GST polymorphisms on breast cancer risk. Bosetti et al. (12) also found a significant association between higher cruciferous vegetable consumptions and lower breast cancer risk compared with no/occasional consumption. The remaining three studies are prospective. Two were based on the Nurses' Health Study, with an earlier report in 2016 showing that vegetable intake, including cruciferous vegetables during adolescence and early adulthood, was significantly associated with a reduced breast cancer risk (28). A 2019 report showed that consuming more than five servings per week of cruciferous vegetables in adulthood was associated with a 9% decreased risk of invasive breast cancer, compared with less than two servings per week (29). Another prospective study conducted in Japan showed that cruciferous vegetable intake was statistically significantly associated with a reduced risk of premenopausal breast cancer, and was marginally inversely associated with ER+PR+ (estrogen receptor positive, progesterone receptor positive) tumors (103). These studies reaffirm the potential benefits of cruciferous vegetable consumption in reducing breast cancer risk, as seen in past observational studies. A more recent meta-analysis summarizing both case-control and cohort studies from 1978 to 2023 further confirmed the significant inverse association between cruciferous vegetable intake and breast cancer risk, showing a 20% decreased risk overall (133). However, it found no overall associations in cohort studies, except for those with a follow-up period over 20 years, indicating a 7% lower incident breast cancer risk (133). This suggests that longer-term consumption of cruciferous vegetables is beneficial.

In contrast, the effect of cruciferous vegetable intake on breast cancer survival remains controversial (74) (**Table 2**). While a significant inverse association exists with incident breast cancer risk, results from the Nurses' Health Study showed that higher intake of cruciferous vegetables after breast cancer diagnosis was not associated with breast cancer–specific mortality. However, there was a significant association between cruciferous vegetable intake and all-cause mortality,

Table 2 Cruciferous vegetables and breast cancer risk and prognosis

Reference	Study design	Total participants	Exposure	Primary outcome(s)	Results
Breast cancer risk					
Zhang et al. 2020 (132)	Case-control	737 breast cancer cases and 756 controls	Cruciferous vegetable, GLS, and ITC intakes; GST polymorphisms	Breast cancer risk	Low intake of cruciferous vegetables, GLS, or ITC was associated with a higher risk of breast cancer. No significant interactions with GST polymorphisms.
Farvid et al. 2019 (29)	Prospective cohort	182,145 women aged 27–59 at enrollment with 10,911 invasive breast cancer cases during follow-up	Cruciferous vegetables	Invasive breast cancer risk	>5 versus 2 servings/week of cruciferous vegetable intake HR (95% CI) = 0.91 (0.85–0.96). A higher intake of total fruits and vegetables also linked to lower risk.
Zhang et al. 2018 (131)	Case-control	A total of 1,485 cases and 1,506 controls were recruited from June 2007 to March 2017	Cruciferous vegetables, GLS, and ITC intakes	Breast cancer risk	Highest quartile versus lowest: OR = 0.51 (95% CI = 0.41, 0.63) for cruciferous vegetables, OR = 0.54 (95% CI 0.44, 0.67) for GLS, and OR = 0.62 (95% CI 0.50, 0.76) for ITC.
Farvid et al. 2016 (28)	Prospective cohort	90,476 premenopausal women aged 27–44 from the Nurses' Health Study II	Vegetables including cruciferous vegetables	Breast cancer risk	Greater consumption of apple, banana, and grapes during adolescence and oranges and kale during early adulthood was significantly associated with a reduced risk of breast cancer.
Suzuki et al. 2013 (103)	Prospective cohort	47,289 Japanese women; during an average of 10.2 years of follow-up, 452 cases of breast cancer were newly diagnosed	Vegetables including cruciferous vegetables	Breast cancer risk	Cruciferous vegetable intake was associated with a decreased risk of premenopausal breast cancer and a marginally inverse association with ER+PR+ tumors. No overall association was present for postmenopausal breast cancer.
Bosetti et al. 2012 (12)	Case-control	The studies included a total of 3,034 of the breast cancer cases and 3,392 controls from a network of case-control studies conducted in Italy and Switzerland	Cruciferous vegetables	Breast cancer risk	Cruciferous vegetable consumption at least once a week versus no/occasional consumption was associated with a significantly reduced breast cancer risk [OR = 0.83 (95% CI = 0.74–0.94)].

(*Continued*)

Table 2 (*Continued*)

Reference	Study design	Total participants	Exposure	Primary outcome(s)	Results
Breast cancer mortality					
Zainordin et al. 2020 (129)	Cross-sectional	77 (50.7 +/− 7.8 years old) breast cancer patients	Increased cruciferous vegetable intake (46.8%) along with increased green leafy vegetable intake	Quality of life	Positive dietary changes were associated with improved emotional functions and reduced fatigue.
Farvid et al. 2020 (30)	Prospective	8,927 stage I–III breast cancer patients from the Nurses' Health Study I (1980–2010) and II (1991–2011) with documented 2,521 deaths, including 1,070 attributed to breast cancer	Cruciferous vegetables	All-cause mortality, breast cancer–specific mortality	Higher cruciferous vegetable intake was associated with lower all-cause mortality: quintile 5 versus 1 HR (0.95% CI) = 0.87 (0.76–0.99), no significant results for breast cancer–specific mortality.
Nechuta et al. 2013 (73)	Prospective	11,390 women from the After Breast Cancer Pooling Project with stage I–III invasive breast cancer; after a median follow-up of 9.0 years, 1,725 deaths and 1,421 recurrences were documented	Cruciferous vegetable intake	Breast cancer outcomes (recurrence and mortality)	No association was found with breast cancer outcomes.
Nomura et al. 2018 (75)	Cross-sectional	192 Chinese American and 173 non-Hispanic White female breast cancer survivors (stages 0–III) who had completed primary treatment	Cruciferous vegetable intake	Breast cancer treatment-related symptoms	Higher cruciferous vegetable intake was associated with lower odds of menopausal symptoms ($\geq$70.8 versus <33.0 g/day, OR = 0.50, 95% CI = 0.25, 0.97).

Abbreviations: CI, confidence interval; ER, estrogen receptor; GLS, glucosinolate; GST, glutathione S-transferase; HR, hazard ratio; ITC, isothiocyanate; OR, odds ratio; PR, progesterone receptor.

showing a 13% decreased risk comparing the highest quintile to the lowest (30). Additionally, the After Breast Cancer Pooling Project found no association between postdiagnosis cruciferous vegetable intake and total mortality or breast cancer recurrence (73). A recent meta-analysis summarizing three cohort studies also confirmed the null association between cruciferous vegetables and breast cancer mortality (107). In terms of quality of life postdiagnosis, a small cross-sectional study (n = 77) reported a positive association between cruciferous vegetable intake and improvement in emotional function and reduction in fatigue symptoms (129). Among breast cancer survivors, a cross-sectional study in Chinese American ($n = 192$) and non-Hispanic White patients ($n = 173$) showed higher cruciferous vegetable intake was associated with a reduced risk of experiencing menopausal symptoms (75). Overall, the evidence does not support the idea that cruciferous vegetable intake improves breast cancer prognosis, suggesting that a targeted intervention approach may offer more tangible benefits to breast cancer patients.

Clinical Trials on Cruciferous Vegetables and Derived Compounds and Breast Cancer Biomarkers

In addition to observational studies, clinical trials have investigated the role of cruciferous vegetables and their derived compounds on breast cancer prevention (**Table 3**). The primary intervention agents include ITC-rich broccoli sprout extract (BSE) (117), DIM supplement (104, 128), GFN supplement (6) or overall cruciferous vegetable intake (119), with the intervention duration ranging from 2 weeks to 1 year among $n = 23$ to $n = 130$ participants. The cancer-related biomarkers of interest assessed in these trials included blood, urine, or imaging biomarkers. Almost all clinical trials have identified significant associations between the intervention agents and the respective biomarkers. For instance, a 2-week intervention with dietary ITC-rich BSE (200 μmol/day) showed an increased trend in cleaved caspase 3 and tumor-infiltrating lymphocytes, along with decreased trends of Ki-67 and nuclear to cytoplasm ratio of estrogen receptor-α, indicating the beneficial effects of ITC-rich cruciferous vegetables on breast cancer prognosis (117). Additionally, a 1-year supplementation of DIM (oral DIM 100 mg/day) led to a significant reduction in fibroglandular tissue, an indicator of percent mammographic density, assessed from magnetic resonance imaging (128). Another 1-year intervention of DIM (150 mg twice/day) increased the 2/16α-OHE1 ratio and serum sex hormone binding globulin level (104), suggesting its beneficial effect on estrogen metabolism. A 3-week intervention of cruciferous vegetable intake (≥14 cups/week) had significantly lower urinary 8-hydroxy-2′-deoxyguanosine levels, a biomarker of oxidative stress (119). Further, a 2–8 week intervention of ~250 mg of a broccoli seed extract containing GFN (BroccoMax^{TM}) (2 pills 3x/day) was associated with a decrease in peripheral blood mononuclear cell HDAC activity, suggesting the effects of SFN in epigenetic regulations (6). Collectively, these studies show an improvement in breast cancer biomarkers with cruciferous vegetable–derived compounds. However, it remains to be seen if these targeted interventions translate to improved prognosis and survival outcomes.

Recent Preclinical Studies on Cruciferous Vegetable–Derived Bioactive Metabolites and Breast Cancer (2018–2024) and Mechanisms of Action

The chemopreventive mechanisms of action of SFN and I3C/DIM, especially with results coming from preclinical models, have been extensively reviewed elsewhere (1, 4, 22, 47, 51, 76, 85, 118) and encompass preclinical evidence demonstrating the ability of SFN and I3C/DIM in inhibiting tumor initiation, progression, and migration/invasion (**Figure 1**). Despite their chemopreventive efficacy in preclinical models, it is unlikely that SFN or I3C/DIM would be used alone as a sole intervention when standard cancer treatments are available. However, increasing evidence in preclinical studies indicates SFN and I3C/DIM may complement and/or enhance conventional cancer therapy, which is the focus of this section. The potential efficacy of SFN and I3C/DIM in preventing tumor progression and migration/invasion is of particular relevance in the context of clinical settings where patients have established tumors, as it may offer a complementary therapeutic strategy in addition to the standard cancer regimen. In breast cancer therapies, drug toxicity (including hematological and nonhematological toxicities) and the development of drug resistance that results in tumor recurrence and relapse are both barriers to effective cancer treatment

Table 3 Clinical trials on cruciferous vegetable–derived compounds and breast cancer

Reference	Total participants	Intervention	Duration	Primary outcomes	Results
Wang et al. 2022 (117)	30 postmenopausal patients diagnosed with breast cancer (mean age 61.2 years)	ITC-rich BSE (200 μmol ITC/day) or placebo	2 weeks	Biomarkers related to ITCs in breast tissue	Trends of increased cleaved caspase 3 and tumor-infiltrating lymphocytes, and decreased Ki-67 and nuclear-to-cytoplasm ratio of ER-α in the BSE arm only
Yerushalmi et al. 2020 (128)	23 healthy female BRCA carriers (median age 47 years; 78% postmenopausal)	DIM, single arm oral DIM 100 mg × 1/day	1 year	FGT and BPE measured by MRI	1-yr DIM supplementation significantly reduced FGT observed on MRI
Thomson et al. 2017 (104)	Breast cancer patients prescribed with tamoxifen ($n = 130$)	Randomly assigned oral BR-DIM at 150 mg twice daily or placebo, for 12 months	1 year	Change in urinary 2/16α-OHE1 ratio; changes in 4-OHE1, serum estrogens, SHBG, breast density, and tamoxifen metabolites	BR-DIM increased the 2/16α-OHE1 ratio (+3.2) versus placebo (−0.7, $P < 0.001$), increased serum SHBG, no change in breast density, and reduced plasma tamoxifen metabolites ($P < 0.001$)
Wirth et al. 2017 (119)	69 healthy postmenopausal women assigned to either control ($n = 34$) or intervention ($n = 34$) group	Cruciferous vegetables (≥14 cups/week) or control	3-week period	Urinary oxidative metabolites	Lower postintervention urinary 8-hydroxy-2′-deoxyguanosine in breast cancer group (1.1 ng/ml versus 3.2 ng/ml, $P = 0.01$) after adjustment
Atwell et al. 2015 (6)	54 women with abnormal mammograms and scheduled for breast biopsy	Intervention: ($n = 27$) ∼250 mg of a broccoli seed extract containing GFN (BroccoMax™) (2 pills 3x/day) versus control ($n = 27$)	2- to 8-week	Plasma and urinary SFN metabolites, PBMC HDAC activity, and tissue biomarkers (H3K18ac, H3K9ac, HDAC3, HDAC6, Ki-67, p21)	GFN supplementation significantly decreased PBMC HDAC activity ($P = 0.04$)

Abbreviations: 2/16α-OHE1, 2/16α-hydroxyestrone; 4-OHE1, 4-hydroxyestrone; BPE, background parenchymal enhancement; BR-DIM, BioResponse DIM; BSE, broccoli sprout extract; DIM, 3,3′-diindolylmethane; ER, estrogen receptor; FGT, fibroglandular tissue; GFN, glucoraphanin; HDAC, histone deacetylase; ITC, isothiocyanate; MRI, magnetic resonance imaging; PBMC, peripheral blood mononuclear cell; SFN, sulforaphane; SHBG, sex hormone binding globulin.

(17, 31, 33, 67, 84). Combination therapies (combining two or more therapeutic agents) have been shown to improve patient outcomes (27, 31). While phytochemical-based therapy is unlikely to replace standard cancer treatments, integrating them has gained interest as a strategy to enhance conventional cancer therapies, potentially offering additive or synergistic effects. Breast cancer animal models and cell culture models have explored the use of SFN and I3C/DIM with various cancer treatments, focusing on the synergism of these combinations in improving treatment efficacy, reducing toxicity, and overcoming resistance. **Table 4** lists recent preclinical studies that investigated the efficacy and mechanisms of protection of SFN or I3C/DIM treatments in

Table 4 Combination SFN/DIM and other anticancer drugs/therapies

Reference	Cancer therapy type	Treatment combinations	Preclinical models	Overall findings
Hajra et al. 2018 (38)	Chemotherapy	I3C 20 mg/kg and/or DOX 5 mg/kg	In vivo: Ehrlich ascites carcinoma bearing mice	I3C enhanced DOX sensitivity by inhibiting NF-κβ, blocking angiogenesis and regulating mitochondrial apoptotic pathway, reducing cardiac toxicities and inflammation.
Bose et al. 2018 (11)	Chemotherapy	In vitro: 2.5 μM SFN and/or 5 μg/ml DOX In vivo: SFN 4 mg/kg and/or DOX 5 mg/kg	In vitro: rat cardiomyocytes, MCF 10A, MCF-7, MAT B III, MDA-MB-231 cells In vivo: rat MAT B III syngeneic tumors	SFN acted synergistically with DOX in cancer regression, reduced DOX dosage required. Protected against DOX-induced toxicity in normal but not cancer cells.
Rong et al. 2020 (86)	Chemotherapy	In vitro: 40 μM SFN and/or 50 nM DOX In vivo: SFN 4 mg/kg and/or DOX 5 mg/kg	In vitro: 4T1 and DOX-resistant 4T1 cells In vivo: mouse 4T1 syngeneic tumors	SFN inhibited MDSCs via reducing PGE2 secretion of breast cancer cells and enhanced DOX efficacy in vivo, resulting in decrease in tumor volume, MDSC expansion, and increase in cytotoxic CD8+ T cells.
Yang et al. 2018 (126)	Chemotherapy	In vitro: 10 μM SFN and/or 1 μM DOX In vivo: SFN 50 mg/kg and/or DOX 2 mg/kg	In vitro: TNBC MDA-MB-231, BT549, and MDA-MB-468 cells In vivo: Mouse MDA-MB-231 xenografts	SFN induced autophagy by inhibition of HDAC6-mediated PTEN activation and enhanced sensitivity to DOX. SFN and DOX combination synergistically inhibited TNBC cell growth in vitro and in vivo.
Mielczarek et al. 2019 (68)	Chemotherapy	0.4–12 μM SFN, 0.01–0.8 μM DOX in liposomes	In vitro: MCF-7, MDA-MB-231 cells	SFN enhanced intracellular accumulation and anticancer action of DOX. A synergistic effect allowed for dose reduction to decrease DOX-mediated toxicity.
Sinha et al. 2019 (97)	Chemotherapy	5 μM SFN and/or 10 μM CP	In vitro: MDA-MB-231 and MDA-MB-468 cells	SFN-CP inhibited the stemness and metastatic potential of TNBCs via down regulation of sirtuin-mediated EMT signaling axis.
Xu et al. 2019 (124)	Chemotherapy	In vitro: SFN and/or CP 10–160 μM In vivo: SFN 0.59 mg/kg and/or CP 2mg/kg	In vitro: MCF-7 cells In vivo: mouse MCF-7 xenograft model	SFN-CP increased cellular uptake and cytotoxicity, increased platinum–DNA complex, and improved CP chemosensitivity by glutathione depletion.
Lanza-Jacoby & Cheng 2018 (50)	Chemotherapy	DIM + docetaxel: 25/50 μM DIM and/or 1 nM DOC	In vitro: MDA-MB231 and Sk-BR-3 cells	DIM enhanced DOC sensitivity by increasing ROS and led to decreased cell survival and apoptosis, and potential for reducing toxicity.
Xiang et al. 2021 (123)	Chemotherapy	30–80 μM DIM and/or 25/50 μM PTX	In vitro: MCF-7 and T47D cells	DIM enhanced PTX sensitivity by suppressing DNMT1-mediated KLF4 methylation, increasing the expression of KLF4.

(*Continued*)

Table 4 (*Continued*)

Reference	Cancer therapy type	Treatment combinations	Preclinical models	Overall findings
Milczarek et al. 2018 (69)	Chemotherapy	5–40 μM SFN and/or 6–50 μM 5-FU	In vitro: MDA-MB-231 cells	SFN and 5-FU acted synergistically by inducing autophagic cell death and premature senescence.
Penta et al. 2021 (80)	Endocrine therapy	25/50 μM DIM and/or 2.5/5 μM CC	In vitro: MDA-MB-231, MDA-MB-468, MCF-7, 4T1 cells	DIM modulated the expression of drug efflux transporters and enhanced the efficacy of CC by retaining intracellular CC concentration.
Penta et al. 2023 (81)	Endocrine therapy	10 mg/kg DIM and/or 2.5 mg/kg CC	In vitro: mouse 4T1 syngeneic tumor model	DIM enhanced the therapeutic effect of CC by suppressing primary tumor growth and metastasis by inhibiting neoangiogenesis and reversing EMT.
Simoes et al. 2020 (95)	Endocrine therapy	In vitro: 5 μM SFX-01 and/or 1 μM TAM In vivo: 300 mg/kg SFX-01 and/or 10 mg/kg TAM or 200 mg/kg fulvestrant	In vitro: patient-derived breast cancer cells In vivo: PDX xenograft model	SFX-01 (stabilized formulation of SFN) used in combination with endocrine therapies prevented CSC enrichment and inhibited CSC-mediated endocrine resistance vis STAT3 signaling.
Sun et al. 2022 (102)	Immune checkpoint inhibitors	10 mg/kg DIM and/or 0.25 mg anti-PD-1	In vivo: mouse 4T1 syngeneic tumor model	DIM improved antitumor immune responses of PD-1 blockade via inhibiting MDSC and promoting T cell response, and inhibited MDSC expansion via the STAT3 signaling pathway.
Li et al. 2021 (53)	Radiation therapy	DIM 75 mg/kg DIM and/or 6 Gy/d	In vivo: E0771 syngeneic tumor model, MDA-MB-231 and HCC1937 xenograft model	DIM enhanced tumor regression in immune-competent but not immune-deficient mice after radiation therapy, increased intratumoral immune infiltrates, enhanced antitumor immune responses, and protected normal cells from radiation-induced immediate injuries.

Note that cell culture studies only list the combo dose that shows synergy where appropriate. Abbreviations: 5-FU, 5-fluorouracil; CC, centchroman; CP, cisplatin; CSCs, cancer stem cells; DIM, 3,3′-diindolylmethane; DOC, docetaxel; DOX, doxorubicin; EMT, epithelial-mesenchymal transition; HDAC, histone deacetylase; I3C, indole-3-carbinol; MDSC, myeloid-derived suppressor cells; PR, progesterone receptor; PTEN, phosphatase and tensin homolog; PTX, paclitaxel; ROS, reactive oxygen species; SFN, sulforaphane; SHBG, sex hormone binding globulin; TAM, tamoxifen; TNBC, triple negative breast cancer.

combination with various cancer therapies (chemotherapy, endocrine therapy, immune checkpoint inhibitors, and radiation therapy), including the range of dosages of SFN and I3C/DIM tested that showed synergy in combination treatments. SFN and I3C/DIM can improve therapeutic efficacy through various mechanisms, broadly categorized as reducing chemotherapy and radiation therapy toxicity while selectively protecting normal cells; increasing drug sensitivity, cytotoxic responses, and intracellular drug accumulation; reducing metastatic potential by inhibiting CSCs and EMT; and promoting antitumor immune response and inhibiting myeloid-derived suppressor cells (MDSCs).

Protect normal cells and reduce treatment toxicity. Chemotherapy drugs such as doxorubicin (DOX) are effective for breast cancer but can cause chronic cardiotoxicity and multidrug resistance that lead to poor prognosis and survival (21, 84). As an anthracycline chemotherapy drug, DOX-induced cardiotoxicity is associated with increased inflammation, oxidative stress, and autophagy (24, 84). Preclinical studies in breast cancer cell lines and animal models suggest that I3C (38) and SFN (11) can protect against DOX-induced toxicities by reducing cardiac oxidative stress (via activation of Nrf2 and antioxidant enzymes) and inflammation (via downregulation of NF-kβ, iNOS, COX-2 and IL-6 in cardiac tissues) and enhancing mitochondrial respiration in the hearts of DOX-treated rats (11, 38). In radiation therapy, while ionizing radiation induces DNA damage in tumor cells, the radiation toxicity also affects normal cells (113). DIM has shown protective effects against radiation-induced DNA damage and oxidative stress in noncancer animal models (26, 64). In breast cancer models, DIM protects against radiation-induced injuries in normal cells but not tumor cells, where DIM-conditioned normal cells are less responsive to radiation-induced gene expression modulation (53).

Increase drug sensitivity, accumulation, and cytotoxicity. In addition to reducing toxicity, the use of phytochemicals in conjunction with cancer therapy has been explored as a strategy to potentiate the efficacy of anticancer drugs. Combining the chemopreventive effects of SFN or DIM (inhibiting cancer cell proliferation, reducing cellular migration and invasion, and inducing cell cycle arrest and apoptosis, among others) with conventional cancer therapies can potentially improve drug cytotoxicity and sensitivity, and reduce drug resistance. In breast cancer models, combinations of SFN or DIM with various chemotherapy drugs have shown synergistic effects in increasing anticancer response in breast cancer cells with mechanisms including increased cellular uptake and drug accumulation in cancer cells, in part by modulating the drug efflux transporters, allowing for the use of lower doses of chemotherapy drugs for the same efficacy with reduced drug-induced toxicity (11, 68, 80). Increased chemosensitivity can enhance drug accumulation, mediated by glutathione depletion (124); increase cytotoxicity via enhanced ROS production (52); upregulate the expression of the tumor suppressor KLF4 (123); and induce autophagy and autophagic cell death (69, 126). This is further demonstrated in animal models, where combination treatments with SFN or DIM result in enhanced tumor regression compared with either treatment alone.

Inhibit epithelial-mesenchymal transition, cancer stem cells, and metastatic potential. Resistance to chemotherapy drugs results in cancer recurrence and is associated with poor prognosis and survival, with 90% of chemotherapy failures that result in cancer invasion and metastasis attributed to drug resistance (67, 83). EMT is a process in which epithelial cells acquire mesenchymal traits, a biological process in normal embryonic development and tissue regeneration. CSCs are a subset of tumor cells that possess stem cell–like characteristics, including self-renewal and differentiation potential. Aberrant reactivation of EMT in primary tumors and increase in CSCs both contribute to therapy resistance, tumor progression, and metastasis (42, 58, 70). Both SFN and I3C/DIM have been shown to inhibit CSCs and the EMT process (22, 39, 89, 111) and can potentially be used to counter development of chemotherapy drug resistance. In breast cancer models, combination therapy of SFN or DIM with chemotherapy and endocrine therapy drugs has been shown to suppress EMT. This is accomplished via downregulation of sirtuin-mediated EMT signaling and downregulation of FAK/MMP9/2 signaling in metastatic breast cancer cells, inhibition of angiogenesis, and downregulation of VEGF signaling, resulting in suppression of lung metastasis in animal models (81, 97). SFN in combination with endocrine therapy has also been shown to inhibit breast CSCs in breast cancer patients' samples and prevent tamoxifen-mediated enrichment for cells with CSC properties and lung metastases in a xenograft model via suppression of STAT3 signaling (95).

Enhance antitumor immune response. Antitumor immunity encompasses both adaptive and innate immune response directed against tumors. Effectiveness of antitumor immunity is hampered by immunosuppressive tumor microenvironment that allows for tumor evasion, as well as immune suppression associated with conventional chemotherapy drugs (8, 71, 121). Both SFN and DIM have been reported to elicit protective immune effects in preclinical studies, including murine models of colitis, leukemia, and experimental autoimmune encephalomyelitis (35, 43, 46, 91). In the context of breast cancer, SFN and DIM enhance antitumor immune response by inhibiting the accumulation, expansion, and activities of immune-suppressive MDSCs, in part via inhibition of prostaglandin E2 synthesis (86), downregulation of miR-21 level (102), and modulation of STAT3 signaling in breast cancer cells (102). SFN and DIM also enhance antitumor immunity by promoting cytotoxic CD8 T cell response and increasing intratumoral immune infiltrates, resulting in improved response to radiation therapy (53) and anti-PD-1 immunotherapy (102).

Synergistic Effects of SFN and DIM with Other Phytochemicals in Breast Cancer

The efficacy of SFN and DIM with other phytochemicals has been investigated in breast cancer cell culture and animal models to determine synergistic effects in reducing breast cancer development and growth. Studies show that the combination of SFN or broccoli sprouts with green tea polyphenols (2), genistein (79, 90), sodium butyrate (90), or withaferin A (82) and the combination of I3C and luteolin (116) enhance the suppression of breast cancer progression. In particular, green tea polyphenols, genistein, sodium butyrate, and withaferin A, when used in combination with SFN, have a greater impact on inducing epigenetic modification than individual phytochemicals administered alone. Transgenerational studies further demonstrate SFN's protective effects via epigenetic regulation, where maternal exposure to broccoli sprouts in the diet protects offspring against mammary cancer formation (3, 54, 56). Moreover, in a recent study, it was observed that the transgenerational protective effects extend to paternal diet exposure when broccoli sprouts are used in combination with green tea polyphenols in a transgenic breast cancer mouse model (55).

Collectively, preclinical evidence suggests that incorporating SFN and I3C/DIM, and other bioactive phytochemicals, into conventional cancer therapies may improve treatment tolerance and efficacy through multiple mechanisms. However, the translatability of these preclinical findings can be limited by the use of phytochemical dosages in some studies that are not achievable in human studies. The physiological relevance of preclinical data will need to be carefully considered prior to the establishment of clinical studies to confirm these observations and determine their impact on breast cancer prognosis and survival.

THE ROLE OF THE MICROBIOME IN BREAST CANCER AND CRUCIFEROUS VEGETABLE CHEMOPREVENTION EFFICACY

Gut Microbiome and Breast Cancer Development, Progression, and Treatment Response

The gut microbiota, which constitutes the bulk of the overall human microbial load and is populated by bacteria, viruses, fungi, and protozoa, plays a key role in breast cancer tumorigenesis and progression, and it impacts breast cancer prognosis and treatment efficacies (**Figure 2**) (10). A few studies have shown that the gut microbiome differs significantly in patients with or without breast cancer or with different stages of breast cancer (36, 66, 134). At the same time, chemotherapy has been shown to alter gut microbiome composition (120). Accumulating evidence has demonstrated the potential mechanisms through which the gut microbiome modulates breast cancer

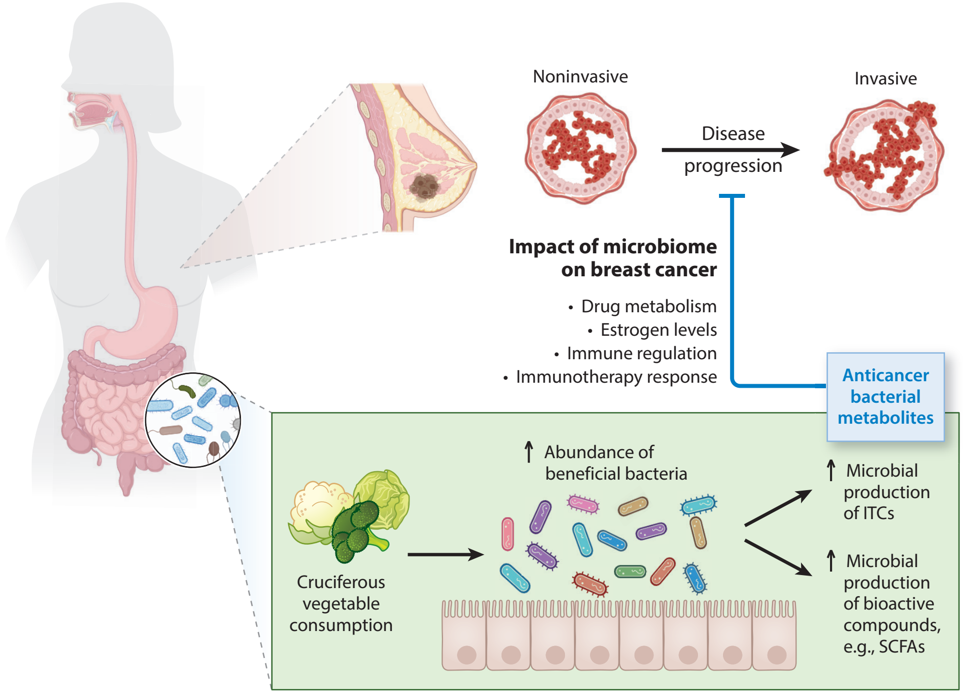


Figure 2

The role of microbiome in breast cancer and cruciferous vegetable chemoprevention efficacy. This figure illustrates the interplay between cruciferous vegetables, derived bioactive compounds, and the gut microbiome. Cruciferous vegetable consumption increases the abundance of beneficial bacteria, which enhance the production of bioactive compounds such as isothiocyanates (ITCs) and short-chain fatty acids (SCFAs). These bacterial metabolites play an important role in breast cancer prevention and treatment response by modulating disease progression, drug metabolism, estrogen levels, the immune system, and immunotherapy response. Figure adapted from images created in BioRender; Wong C. 2024. **https://BioRender.com/p72l288**.

progression. These include but are not limited to modulation of immune system activity, alteration of estrogen levels, and production of pro- and anticancer bacterial metabolites, all of which collectively influence both cancer and immune cells in the breast tumor microenvironment (10).

Microbiome can also influence treatment outcomes by metabolizing chemotherapeutic and hormonal drugs, and it can modulate the effectiveness of tumor immunotherapies by regulating antitumor immunity (98, 112). Studies have shown that immunotherapy response is closely related with native gut microbiota signatures and dietary intake (96), and modifying dietary intake (e.g., dietary fiber, choline, fat) can alter microbiome composition, microbial metabolite profile, and tumor immunity (19, 100, 112). The emerging interaction between diet, gut microbiome composition, and breast cancer suggests the potential to enhance immunotherapy outcomes in breast cancer patients through microbiota and dietary modulation (109, 115).

Cruciferous Vegetable Consumption and Gut Microbiome in Breast Cancer Prevention

High interindividual variation in both the efficacy of and response to cruciferous vegetables is commonly observed in human clinical trials. Increasing evidence suggests that variations in microbiome composition may play a role in driving such interindividual variations, with the potential to influence chemopreventive efficacy of cruciferous vegetables on breast cancer development and treatment response. Mechanistically, the gut microbiome may alter efficacy and risk through the production of SFN (14, 15, 40, 61, 94, 106), short chain fatty acids (SCFAs) (37, 92, 99, 127), and other potentially bioactive products (**Figure 2**) (13, 65). Additionally, cruciferous vegetable consumption may alter gut microbiome composition, modulating risk and response to treatment (9, 18, 63, 92, 122). The individual's microbiome can influence GLS metabolism (14), as numerous studies have found human gut microbiome providing myrosinase activity, thereby enhancing the beneficial effects of consuming GLS-rich vegetables (94). Work conducted in vivo and in vitro has shown a wide range of phylogenetically diverse bacteria can metabolize GLS to ITCs, suggesting it is a trait found widely across microbiomes (14, 15, 40, 61, 106). Work conducted in vitro has demonstrated that gut microbiome composition dictates differences in nitrile production from GLSs, thus suggesting microbiome composition can directly influence bioavailability of ITCs from GLSs, and potentially shifts the balance between the production of bioactive ITCs and biologically inert nitriles (13, 65).

While the role of the gut microbiome in ITC or other microbial metabolite generation and cancer incidence and progression are still being elucidated, the gut microbiome offers an exciting new frontier in cancer prevention. The inherent ability of the microbiome to adapt and change with alterations in local microenvironment offers opportunities to influence its composition via exposure to bioactive ITCs. Prolonged consumption of cruciferous vegetables has been shown in rodents to improve the conversion of GLS to ITCs by gut microbes (63, 122). In fact, the genes underpinning GLS hydrolysis in *Bacteroides thetaiotaomicron* are also responsible for glycan hydrolysis, suggesting dietary factors beyond cruciferous vegetables may alter their expression, and thus GLS-hydrolyzing potential, in these microbes (61). Ex vivo human fecal incubation work has shown that differences in gut microbiome composition influence the production of GLS-derived nitriles from broccoli sprouts (13). These findings support the notion that variations in gut microbiome composition influence interindividual variation in the production of bioactive compounds from cruciferous vegetables and thus the efficacy of whole food interventions. In addition to ITCs, the composition of the microbiome influences other microbially derived compounds from cruciferous vegetables, including the production of SCFAs such as butyrate, a microbial metabolite of dietary fiber that is a known HDAC inhibitor and has anti-inflammatory and immunomodulatory properties (15, 37, 92, 99, 127). These compounds may act synergistically to ITCs to play a role in cancer prevention.

Work in both humans and rodent models has also shown that prolonged consumption of cruciferous vegetables alters gut microbiome composition (63). Various studies have shown that the same set of microbes reported to be altered by exposure to broccoli or ITCs were also associated with responsiveness to DOX treatment or breast cancer status. For example, in an animal study, mice that responded positively to DOX had increased abundance of *Akkermansia muciniphila* and *Roseburia intestinalis* while those that did not respond had increased abundance of *Alistipes putredinis* (9). Interestingly, changes in the abundance of *A. muciniphila*, *R. intestinalis*, and *A. putredinis* have been shown to be influenced by broccoli and ITC exposure. Work in rats has shown that consumption of broccoli leads to an increased abundance of *A. muciniphila* (130), and a feeding study conducted in humans found a positive correlation between excretion of ITCs and the abundance of a member of *Roseburia*, while a negative relationship was observed between excreted ITCs and *A. putredinis* and *Blautia* (16). Studies have found an increase in members of *Blautia* in breast cancer patients compared with healthy controls (92) and an increase in members of *Blautia* in more severe breast cancer histoprognosis (66), and rodent studies have shown that prolonged consumption of cruciferous vegetables led to a decrease in the level of *Blautia* in the gut (63). Other studies have indicated that increased consumption of cruciferous vegetables decreases the abundance of sulfate reducing bacteria in the gut, such as *Desulfovibrio* (48). Similarly, *Desulfovibrio* has been shown to be enriched in the gut microbiomes of mice fed a high-fat diet and to produce leucine activating the mTORC1 pathway and increasing breast cancer progression (19).

As summarized above, gut microbiome composition plays a potentially important role in breast cancer risk and incidence, as well as breast cancer patients' treatment outcomes (18). Consumption of cruciferous vegetables may decrease the incidence of breast cancer not only through the production of chemoprotective compounds, such as ITCs, but also by modulating the abundance of cancer-promoting microbes in the gut. These complex interactions between diet, gut microbiome composition, and breast cancer warrant more in-depth investigation. A combination of both systems biology and reductionist approaches is necessary to move beyond associations and discover specific mechanisms through which the gut microbiome modulates breast cancer risk. Additionally, work utilizing metagenomic and metatranscriptomic methods, which reflect not only community composition but also function, is essential for identifying links between cruciferous vegetable consumption and breast cancer prevention.

SUMMARY OF CURRENT EVIDENCE AND CHALLENGES

The current literature has shown promising effects regarding the potential of SFN and I3C/DIM on reducing breast cancer development and progression and improving treatment response. Therefore, recommending dietary consumption of cruciferous vegetables, which generate these ITC compounds within the human body, holds significant implications and promise for public health recommendations. The current dietary guidelines for breast cancer patients recommend consuming 5–9 servings per day of fruits (about 150 g per serving) and vegetables (about 75 g per serving), with a focus on including cruciferous vegetables as a key component of nutrition therapy. Given that there is a considerable variability among individuals' response to cruciferous vegetables and their derived compounds, a one-size-fits-all recommendation may not be appropriate. Factors such as the source and preparation of the food, host genotype, and microbiome composition should all be taken into account to tailor precision nutrition recommendations for each individual.

CONSIDERATIONS FOR FUTURE STUDY DESIGNS

Advances in Bioavailability and Delivery Mechanisms

To gain a deeper understanding of the variations in individuals' response, it is crucial to delve deeper into the key biological, genetic, and metabolic determinants and establish accurate dosing guidelines. SFN, one of the primary derivatives from cruciferous vegetables, has been extensively studied due to its health-promoting effects and low tissue toxicity. However, its bioavailability differs from whole foods and dietary supplements (5). In addition, optimal delivery methods and dosing regimens have yet to be established. Numerous clinical studies and randomized clinical trials have assessed the effects of SFN-related products, ranging from beverages to tablets, at different dosages (25), aiming to maximize its health benefits. The published literature has reported a median effective dose of 175 μmol/kg body weight for SFN administered orally, and 113 μmol/kg for intraperitoneal administration (125). Importantly, a promising advancement in enhancing the efficacy of SFN involves nanoparticle encapsulation. This technology addresses the low solubility of SFN supplements in water and poor oral bioavailability. By doing so, the potential benefits of SFN in cancer therapy can be significantly amplified, as the nanoparticles can form complexes with other molecules such as chemotherapeutic agents, leading to a synergistic antitumor effect (87). For indoles, the dosage of I3C/DIM used in preclinical studies to show chemoprevention efficacy is difficult to achieve using a whole food approach; thus, supplementation with I3C/DIM is likely needed in clinical settings (118). I3C has very low bioavailability after oral ingestion due to its transformation in the gastric acidic environment, including 20–40% conversion to DIM and other acid condensation products (118). Direct supplementation with DIM is preferable, as DIM is not affected by the acidic environment in the stomach, and it circumvents potential problems associated with additional acid condensation products with unknown pharmacological properties. While crystalline DIM is highly insoluble and poorly absorbed upon ingestion, DIM bioavailability is improved with formulation as absorption-enhanced BioResponse DIM, with 50% higher bioavailability compared with its crystalline form (105). Until recently, pharmacokinetic studies only detected parent DIM compounds after DIM supplementation. A recent study reported significant phase 1 and phase 2 metabolism of DIM after oral dosing in humans, with detection of mono- and dihydroxylated metabolites as well as sulfate and glucuronide conjugates in both plasma and urine samples, with one of the metabolites exhibiting greater potency as an aryl hydrocarbon receptor (AhR) agonist (108). Further work is needed to better characterize DIM metabolism and its implication in chemoprevention mechanisms.

Role of Genotyping

Recent evidence suggests that discrepancies in treatment efficacy may be attributed to substantial interindividual variations in the absorption and excretion of bioactive metabolites derived from dietary GLSs. These disparities are influenced, in part, by host genetics, with different polymorphisms in GST contributing to the variation (101), with GSTM1 genotypes exerting a notable influence on the metabolism of SFN derived from both standard and high-GLS broccoli (34). For example, *GSTM1*-positive individuals had a greater SFN metabolism after consuming standard broccoli and high-GLS broccoli compared with *GSTM1*-null individuals (34); in two population-based cohorts in China, it was found that overall urinary ITC levels were not significantly associated with GST gene polymorphisms, but differences by genotype were found among current smokers (32).

Role of the Microbiome in Breast Cancer Outcomes

SFN can significantly modify the gut microbiota composition and vice versa, which can be used to enhance SFN's health benefits. As discussed, gut microbiome variation contributes to the interindividual variation in the efficacy of cruciferous vegetables' health impact on the human body. Human gut microbial communities are altered by the addition of cruciferous vegetables to an established diet (52). While the role of the microbiota in breast cancer is not fully understood, its impact is likely mediated in part via the bacterial metabolites, which can have both pro- and anticancer properties influenced by gut microbiome composition. Furthermore, the gut microbiome interacts with the host systematic immune system, affecting distal organs, including the breast. More work needs to be done to elucidate the interactions between the gut microbiome and immunity, and their influence on breast cancer development and progression.

Role of Breast Cancer Molecular Subtyping and Cancer Stage

The efficacy of SFN in treating breast cancer varies significantly depending on the cancer stage and molecular subtype. Breast cancer is categorized into different molecular subtypes based on the presence or absence of hormone receptors, ER, PR, and human epidermal growth factor 2. The choice of breast cancer therapy is primarily determined by cancer subtype and stage (110), with standard treatments including surgical resection, radiation therapy, endocrine therapy, chemotherapy, and immunotherapy (110). However, drug-induced toxicity and drug resistance can lead to cancer recurrence, poor prognosis, and poor survival (20, 49, 77, 83). Growing evidence from preclinical studies has demonstrated that a combination of SFN or I3C/DIM with commonly used chemotherapy drugs could improve the therapeutic effects. Yet, whether these findings can be translated to human studies remains uncertain. To date, there is only one registered ongoing clinical trial (NCT03934905) that investigates the protective effects of SFN on breast cancer chemotherapy toxicity. Thus, beyond the routine standard treatment, strategies also recommend adequate cruciferous vegetable intake (57); sufficient consumption of both macronutrients and micronutrients can help prevent a decline in the health status of breast cancer patients and improve their cancer prognosis (60).

While a limited number of human studies have explored the relationship between cruciferous vegetable intake and breast cancer molecular subtypes, the findings have been inconsistent. Suzuki et al. (103) reported a marginally inverse association of cruciferous vegetables with ER+PR+ tumors, while Farvid et al. (29) reported an inverse association between broccoli or cauliflower intake and ER– breast cancer, as well as cabbage and cauliflower intake with ER+ breast cancer. The majority of the research on SFN's effect on breast cancer molecular subtypes has been conducted through in vitro studies using cell lines derived from patients representing different subtypes of cancer. Future studies targeted at specific breast cancer subtypes in human subjects are warranted. Also, despite the moderate effect of cruciferous vegetables on breast cancer reported in many earlier epidemiological studies (59), further studies especially incorporating factors impacting interindividual variations in the efficacy and effects of cruciferous vegetables and their derived bioactive compounds are needed.

Synergistic Effects of SFN and DIM in Cancer Prevention

Beyond the use of SFN and I3C/DIM in isolation as cancer prevention, their use in combination has been largely underexplored. SFN's primary route of action is through the induction of Nrf2, leading to increased transcription of the antioxidant response element (44, 88). Nrf2 activation leads to the upregulation of phase II drug-metabolizing enzymes, and DIM/I3C, on the other hand, acts in a complementary manner by binding to AhR, leading to increased transcription of the xenobiotic response element (1, 88, 118). This, in turn, leads to the upregulation of phase I and phase II drug-metabolizing enzymes. Additionally, I3C/DIM can alter estrogen metabolism and block estrogen signaling pathways (1, 118). Despite the apparent synergy, only a handful of in vitro studies have investigated their benefits in combination. One study conducted in HepG2-C8 found that SFN and DIM in combination lead to a synergistic induction of Nrf2 and ARE signaling pathways (88). Another study conducted in the human colon cancer cell line found that a combination of DIM and SFN led to inhibition of cell proliferation (78). While further work is needed to investigate the synergism of these compounds, their use in combination offers a novel area of research within the field of cruciferous vegetable phytochemicals.

DISCLOSURE STATEMENT

E.H. serves on the scientific advisory boards for Haleon, Vytology, and Amway and has served as a paid scientific expert for testimony with Bayer.

AUTHOR CONTRIBUTIONS

E.H., C.P.W., and Z.Z. conducted the primary literature search and collaboratively wrote the first draft of the manuscript. J.A.B. and C.P.W. created the figures and tables and were involved in manuscript drafting. Z.Z. and E.H. supervised the manuscript writing. J.S. reviewed and edited the manuscript. All the authors critically revised the manuscript for important intellectual content.

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