REVIEW

Molecular targets of isothiocyanates in cancer: Recent advances

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Cancer is a multistep process resulting in uncontrolled cell division. It results from aberrant signaling pathways that lead to uninhibited cell division and growth. Various recent epidemiological studies have indicated that consumption of cruciferous vegetables, such as garden cress, broccoli, etc., reduces the risk of cancer. Isothiocyanates (ITCs) have been identified as major active constituents of cruciferous vegetables. ITCs occur in plants as glucosinolate and can readily be derived by hydrolysis. Numerous mechanistic studies have demonstrated the anticancer effects of ITCs in various cancer types. ITCs suppress tumor growth by generating reactive oxygen species or by inducing cycle arrest leading to apoptosis. Based on the exciting outcomes of preclinical studies, few ITCs have advanced to the clinical phase. Available data from preclinical as well as available clinical studies suggest ITCs to be one of the promising anticancer agents available from natural sources. This is an up-to-date exhaustive review on the preventive and therapeutic effects of ITCs in cancer.

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1 Introduction

Cancer is the leading cause of deaths worldwide, accounting for 7.6 million deaths according to recent statistics. The number of deaths due to cancer is projected to increase to 13.1 million in 2030. These figures implicate marginal efficacy of present standard available therapies to cancer patients, implying the urgent need to identify new strategies/agents that can be included in cancer preventive or therapeutic regimen.

Historical evidence purports nature being a prodigious source of many drugs and drug leads for various ailments, including cancer [1]. Several epidemiological studies have been published over the past few decades that indicate a strong correlation between intake of fruits and vegetables and reduced risk of cancer [2–4]. Basic benefits of using bioactive dietary agents are low cost, well-known applications in traditional medicinal system, accessibility, and minimal or no toxicity.

Epidemiological and case–control studies continue to support the notion that consumption of cruciferous vegetables reduces the risk of developing various types of cancers, such as pancreatic, prostate, ovarian, and breast [5–11]. Isothiocyanates (ITCs) occur in cruciferous vegetables as glucosinolates and are converted to ITCs by the action of the enzyme myrosinase. ITCs from these vegetables are also released by cutting or chewing or by intestinal microflora present in humans [12] (Fig. 1). ITCs have been shown to have substantial chemopreventive activity against various human malignancies [13, 14]. Some of the widely studied ITCs that have potent anticancer effects are allyl isothiocyanate (AITC), benzylisothiocyanate (BITC), phenethylisothiocyanate (PEITC), and sulforaphane (SFN). Unless stated, most of the studies mentioned in this article used 95–98% pure ITCs for

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Abbreviations: AITC, allyl isothiocyanate; **BITC**, benzylisothiocyanate; **CYP**, cytochrome P450; **EGFR**, epidermal growth factor receptor; **EGG**, epigallocatechin gallate; **EMT**, epithelial to mesenchymal transition; **FOXO**, forkhead box protein; **GST**, glutathione-S-transferase; **ITC**, isothiocyanate; **PEITC**, phenethyl isothiocyanate; **ROS**, reactive oxygen species; **SFN**, sulforaphane; **STAT3**, signal transducer and activator of transcription 3; **TRAIL**, TNF-related apoptosis-inducing ligand; **VEGF**, vascular endothelial growth factor

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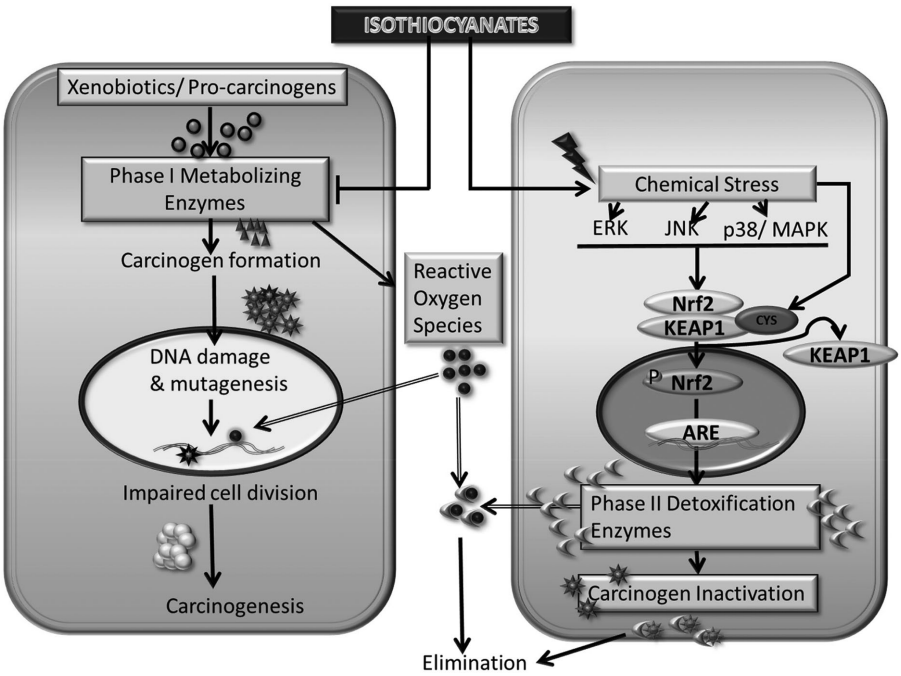

Figure 1. Chemopreventive effects of ITCs.

evaluating anticancer effects. This exhaustive review highlights the specificity of ITCs against various targets in cancer.

2 Chemoprevention by ITCs

An individual's susceptibility to cancer is determined by numerous factors, including maintenance of a critical balance between phase I and II enzymes. Phase I primarily consists of cytochrome P450 enzymes that play an important role in metabolizing the xenobiotics and carcinogens. However, in this process, several chemicals or procarcinogens are activated or converted into highly reactive electrophilic metabolites. The generated electrophiles can disturb the genomic stability by causing DNA damage. Chemopreventive effects of ITCs are exerted by inhibition of the bioactivation of carcinogens by phase I drug metabolizing enzymes (Fig. 1) [15–18]. The mechanistic studies by Morse et al. and others indicate that administration of ITCs prevents the tumor promoting effects of various chemical carcinogens in different animal models [19–21]. PEITC has been shown to possess significant chemopreventive properties against tobacco-induced carcinogens in rodent models of lung and esophageal cancers [22,23]. AITC also inhibits NNK (a tobacco-derived carcinogen) induced tumors in rats [24]. Similar to other ITCs, AITC induces phase II detoxifying enzymes quinone reductase and glutathione-S-transferase (GST) in the animal tissues [25]. Cytochrome P450E1 and *N*-dimethylnitrosoamine demethylase are major enzymes that cause bioactivation of tobacco specific nitrosoamines. These enzymes can be inhibited by glutathione conjugates of ITCs [26]. ITCs also inhibit various isoforms of cytochrome P450 (CYP450) directly, for example, BITC suppresses cytochrome P450 2E1, while SFN inhibits cytochrome P450 1A2 [27, 28]. SFN also has been shown to inhibit steroid and xenobiotic receptor, a nuclear hormone receptor that regulates expression of CYP3A4 [29]. Zhou et al. showed specific antagonism by SFN to inhibit drug clearance due to steroid and xenobiotic receptor induced activity of CYP3A4.

Phase II enzymes, such as GST, NADPH quinine oxidoreductase, and UDP-glucuronosyltransferases play an important role in detoxifying carcinogens as well as xenobiotics. ITCs are known to induce phase II enzymes that further explain the cancer chemopreventive activity of ITCs [28,30–34]. GST catalyzes the conjugation of glutathione with electrophilic compounds making them more water-soluble and facilitating their removal from the body [35, 36]. It is well known that ITC–GSH conjugate is exported out by MRPs [37]. As a result of continuous conjugation and efflux of the conjugate, intracellular GSH level drops significantly within 3 h of ITC treatment. This time also coincides with the induction of GST and mitogen-activated protein kinase [38]. Due to nonavailability of GSH, ITCs bind with other vital cellular proteins causing their thiocarbamoylation [37]. Although being electrophilic, no studies have reported direct binding of ITCs to cellular DNA [39]. In addition, PEITC has been shown to demethylate the promoter region of GSTP1 to induce the expression of GSTP1 [40]. ITCs also induce GSTs that scavenge reactive oxygen species (ROS) [41]. The action of phase II enzymes is primarily regulated by the antioxidant or electrophile response element. The latter can be activated by the transcription factors, such as the basic leucine zipper (bZIP) Nrf2, that heterodimerizes with Maf G/K to exhibit its effects. ITCs induce the Nrf2 transcription factor to activate

antioxidant response element that in-turn translates in the activation of mitogen activated protein kinase ERK/JNK, PI3K, and PKC [41–44]. SFN induces epigenetic modifications by inhibition of histone deacetylase (HDAC) 1, 4, 5, and 7. In addition, SFN-induced demethylation at the promoter region of Nrf2 causes enhanced expression of Nrf2 in the TRAMP mice model for prostate cancer [45]. SFN's chemopreventive effects mainly depend on induction of phase II enzymes through the activation of antioxidant response elements, such as Keap1/Nrf2 [31, 46, 47]. SFN-mediated induction of Nrf2 was found to be through the activation of heme oxygenase 1 and inhibition of p38 in hepatoma cells [42]. Furthermore, several studies have shown induction of thioredoxin reductase as well its substrate thioredoxin by SFN in various cancer cell lines [48–50]. Inhibition of key survival pathway, such as NF-κB and AP-1, by ITCs also contributes to the chemopreventive effects of ITCs [44].

ITCs thus modulate phase I and II enzymes to reduce the bioactivation of carcinogens as well as enhanced detoxification. This dual mechanism leads to reduced binding of carcinogens with the DNA and hence less mutagenic or carcinogenic effects.

These studies suggest existence of mutually distinct mechanisms of chemopreventive and chemotherapeutic effects of ITCs. Specific targets have been identified that mediate chemotherapeutics effects of different ITCs against human cancers [30, 41, 44, 51–53]. These targets might vary with the structural variations among ITCs as well as the nature and origin of cancer. Several studies demonstrate that ITCs modulate cancer cell signaling by acting on multiple targets to suppress growth and progression of cancer cells [41, 53].

3 Uptake of ITCs by cancer cells

The uptake of anticancer agents is an important limiting factor for efficacy. Most of the ITCs can be taken up by the cells through passive diffusion. The cellular uptake of ITCs correlates with the induction of phase II detoxifying enzymes important for chemopreventive activity. It was observed that the intracellular concentrations of ITCs can reach up to 100- to 200-fold higher than the extracellular concentrations. For example, when hepatoma cells were incubated with 100 μM SFN for about 30 min, the intracellular concentrations reached about 6.4 mM [38]. The magnification of intracellular concentration was due to the formation of dithiocarbamates, as ITCs rapidly conjugate with thiols, particularly GSH (Fig. 2). Uptake of ITCs in cancer cells was GSH dependent. The uptake was reduced if GSH concentration was increased. The ITC–GSH conjugate being the substrate of MRPs is transported out of the cells (Fig. 2). This mechanism of uptake and cellular accumulation can be vital in designing the dose regimens of these ITCs. The dose will require the adjustment for high accumulation as well as to compensate for the rapid export through transport proteins, such as MRPs [54]. The shuttling of ITC–GSH causes prompt depletion of intracellular GSH, resulting in the perturbation of cellular redox homeostasis. This could be one plausible mechanism of ROS generation by ITCs.

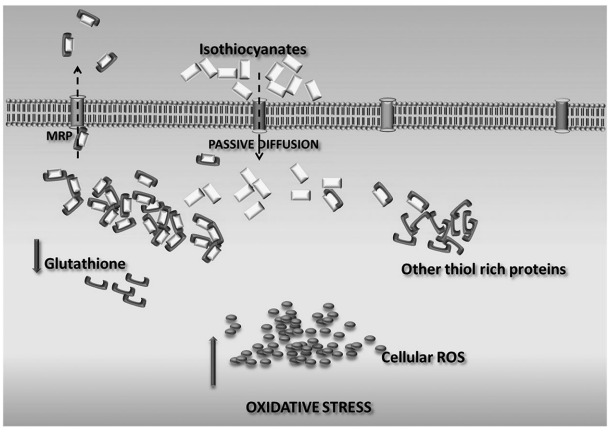

Figure 2. Mechanism of cellular uptake of ITCs.

4 Chemotherapeutic targets

4.1 Benzylisothiocyanate (BITC)

BITC occurs in cruciferous vegetables like cabbage, mustard, watercress, cauliflower, and horseradish that constitute a significant proportion of our daily diet (Fig. 3). Accumulating evidence suggests the anticancer effects of BITC through suppression of initiation, growth, and metastasis of human cancers in various mouse models [55–61]. BITC induces apoptosis selectively in cancer cells through multiple mechanisms [55, 60, 62]. Major anticancer effects of BITC are due to the generation of ROS. BITC causes cell-cycle arrest as well as disruption of mitochondrial membrane potential to initiate mitochondrial pathway of apoptosis [57,63]. Studies from our laboratory have demonstrated the anticancer effect of BITC against pancreatic tumor growth via

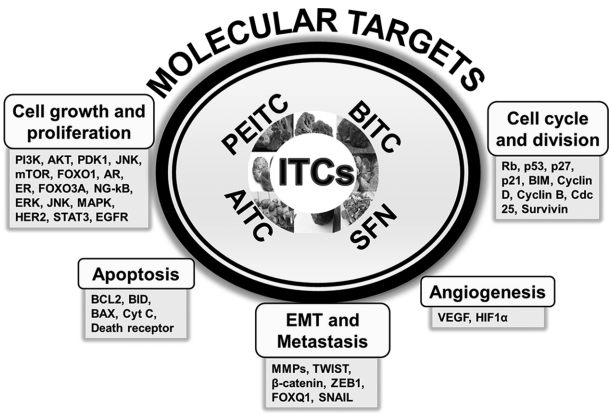

Figure 3. Chemotherapeutic targets of ITCs.

inhibition of key molecules overexpressed in cancer, such as protein kinase B (AKT) signal transducer and activator of transcription 3, HDAC, and nuclear factor kappa B (NFκB) (Table 1) [7, 55, 56, 59]. The targets of BITC can be divided as per their role against cancer-promoting mechanisms.

4.1.1 Cell proliferation and growth

Phosphoinositide 3 kinase (PI3K)/AKT pathway has been shown to be activated in about 59% of the pancreatic tumors, and it also promotes cell division in other cancer forms [64, 65]. PI3K stimulation results in the phosphorylation of AKT at Thr-308 and Ser-473 through phosphatidylinositol-dependent kinase (PDK1) activation [66]. Studies by Boreddy et al. have shown that BITC inhibits PI3K/AKT signaling. BITC prevents the phosphorylation of AKT at both Thr-308 and Ser-473 along with suppression of PI3K (Tyr-458), PDK1 (Ser-241), mammalian target of rapamycin (mTOR) (Ser-2448) [55]. The inhibition of mTOR signaling by BITC was also observed in human prostate cancer cells [67]. These studies showed that BITC had negligible effect on normal human pancreatic ductal epithelial 6 cells, suggesting the specificity of BITC toward cancer cells [55]. These results also showed upregulation of proapoptotic proteins, such as Bim, p21, and p27, due to nuclear accumulation of Forkhead Box Protein 1 (FOXO1). Inhibition of phosphorylation of FOXO1 (Ser-256) and FOXO3a by BITC was due to the dephosphorylation of AKT in pancreatic cancer cells [55]. Interestingly, BITC also reduced acetylation of FOXO proteins by reducing the level of CREB-binding protein (CBP) protein [55]. FOXO1 suppression was also shown to be responsible for BITC initiated cell death in breast cancer cells [68].

NF-κB is a transcription factor that regulates cellular inflammation, immunity, and proliferation [69, 70]. Batra et al. showed that BITC-mediated downregulation of HDAC1 and HDAC3 expression was associated with the acetylation of NF-κB in pancreatic cancer cells [56]. BITC treatment significantly suppressed the phosphorylation of NF-κB at Ser-276 and Ser-536 in BxPC-3 and Capan-2 cells in a dose and time dependent manner [56, 61]. BITC reduced NF-κB protein expression in BxPC-3 cells but not in Capan-2 cells, indicating that BITC acts differentially on different cell lines [56]. The Capan-2 cells have wild-type p53, whereas BxPC-3 cells harbor mutated p53, hence the role of p53 in BITC-mediated downregulation of NF-κB expression cannot be ruled out and remains to be explored further. The mechanistic studies revealed that neither I kappa B (IκB) phosphorylation nor expression levels were altered by BITC, whereas IκB kinase (IKK) expression was downregulated. Hence, downregulation of IKK by BITC treatment could be the reason for inhibition of NF-κB phosphorylation (Ser-536) [56, 61].

STAT3 is hyperactivated in significant number of malignancies, such as breast cancer, pancreatic cancer, gastric cancer, and head and neck cancer as well as in cancer stem cells where it enhances tumor aggressiveness and progression [71–73]. Sahu and Srivastava have shown that BITC suppresses the phosphorylation (Tyr-405 and Ser-727) and expression of STAT3 in pancreatic cancer cells lines, such as BxPC-3, PanC-1, Capan-2, and MIA PaCa-2 [59]. The role of STAT3 in the anticancer effects of BITC was confirmed by STAT3α overexpression or through activation by IL6, which abrogates the effects of BITC (Table 1) [59].

4.1.2 Angiogenesis

The growing tumors are nourished through processes such as angiogenesis and neovascularization. Angiogenesis is mainly promoted by hypoxia inducible factor (HIF-1α) and vascular endothelial growth factor (VEGF) [74]. STAT3 has been shown to be a positive regulator of VEGF and HIF-1α [75, 76]. Boreddy et al. demonstrated that BITC inhibits angiogenesis in chicken chorioallantoic membrane and rat aortic ring assay [77]. This clearly indicates the antiangiogenic potential of BITC. BITC-mediated suppression breast cancer xenografts was associated with inhibition of critical angiogenic factors, such as CD31 and VEGF [78]. Furthermore, BITC downregulated the expression of HIF-1α; vascular endothelial growth factor receptor (VEGFR); MMP-2 (where MMP is matrix metalloproteinase); Rho A; Rho C; and ras-related C3 botulinum toxin substrate 1 (RAC1), 2, and 3 in pancreatic, but the inhibition of VEGF, HIF-1α, and MMP-2 was not observed in STAT3 overexpressing BxPC-3 cells [77]. This undoubtedly suggests that inhibition of tumor growth and angiogenesis by BITC correlates with STAT3 inhibition.

4.1.3 Mitochondrial cell death

Generation of ROS is an important mechanism to induce cell death, specifically in cancer cells. As shown by us and others, BITC significantly induced ROS generation in pancreatic cancer cells and glioma as well as other cancer models [57, 79–82]. ROS generation leads to disruption of mitochondrial membrane potential and release of proapoptotic molecules resulting in activation of caspase-mediated cell death [63, 81, 83]. Furthermore, BITC-mediated downregulation of myeloid cell leukemia marker 1 (MCL-1) in human leukemia cells was also found to be correlated with the mitochondrial pathway of apoptosis [84].

4.1.4 Cell-cycle arrest

ROS induced by BITC also damage DNA and causes G2/M cell-cycle arrest as detected through increased phosphorylation of H2A.X (Ser-139) and ChK2 (Thr-68) [57, 60, 61]. Antioxidants block the effects of BITC confirming the role of ROS in cell-cycle arrest [57]. BITC treatment increased the phosphorylation of the MAP kinases, such as ERK (Thr202/Thy204), JNK (Thr183/Tyr185), as well as p38 (Thr180/Tyr182), in a dose-dependent fashion [57, 62]. It was later found that BITC-induced cell-cycle arrest was executed only through ERK,

Table 1. Chemo preventive effects of ITCs

Compounds	Efficacy	Organ	Mechanism		Dose/duration	Cell line	References
BITC	Antiangiogenesis	Pancreas	↓VEGF, MMP-2, HIF-α ↑RhoB ↓STAT3 phosphorylation (Tyr-705), HIF-α, VEGFR-2, VEGF, MMP-2, CD31, RhoC	In vitro In vivo	5, 10, 20 μM/24 h 12 μmol/40 days	BxPC-3, PanC-1	[77]
BITC	Antiangiogenesis	Breast	↓CD31, VEGF, VEGFR	In vitro In vivo	2.5, 5, 7.5 μM/24 and 48 h 6 and 9 μmol/14 days	MDA-MB-231	[78]
BITC	Apoptosis	Pancreas	↓PI3K, AKT, PDK1, mTOR, FOXO1, FOXO3a ↑Bim, p27, and p21	In vitro In vivo	5, 10, 20 μM/24 h 12 μmol/45 days	BxPC-3, PanC-1	[55]
BITC	Apoptosis	Breast	↓mTOR, FOXO1	In vitro	2.5, 5 μM/6 and 9 h	MDA-MB-231, MCF-7, MDA-MB-468, BT-474, BRI-JM04	[68]
BITC	Apoptosis	Pancreas	↓NF-kappaB, cyclin D1, ↑HDAC1, and HDAC3	In vitro	10 μM/24 h	BxPC-3, Capan-2	[56]
BITC	Apoptosis	Pancreas	↑ERK, JNK, P38, ROS	In vitro	10 μM/24 h	Capan-2, MIA PaCa-2	[57]
BITC	Apoptosis	Pancreas	↑ATR (Ser-428), Chk2 (Thr-68), Cdc25C (Ser-216), Cdk-1 (Tyr-15), and induction of p21Waf1/Cip1 ↓NF-kappa B	In vitro	2.5, 5, 10 μM/24, 48 h	BxPC-3, PanC-1	[58]
BITC	Apoptosis	Pancreas	↓STAT3, MCL-1, BCL-2	In vitro In vivo	5–40 μM/24 h 60 μmol/wk /6 wk	BxPC-3, AsPC-1, Capan-2, MIA PaCa-2, Panc-1	[59]
BITC	Apoptosis, cell-cycle arrest	Brain	↑ROS	In vitro	10, 20 μM/24 h	U87MG	[82]
BITC	Apoptosis	Pancreas	↑H2A.x, p21, Chk2 ↓CyclinB1, Cdc2, Cdc25C	In vivo	10 μmol/L/24 h	Capan-2	[60]
BITC	Apoptosis, cell-cycle arrest	pancreas	↓Cdk1, cyclin B1, Cdc25B, NF-κB ↑IkappaBa	In vitro	5, 10 μM/24 h	BxPC-3	[61]
BITC	Apoptosis	Ovary	↓BCL-2, ERK1/2, and AKT ↑Caspase-3, caspase-9, Bax, JNK1/2, and p38	In vitro	10 μM/48 h	SKOV-3, KLE, SW954, SW756, HL60	[62]
BITC	Apoptosis, cell-cycle arrest	Bone	↓Cyclin A, cyclin B1, ↑Chk1, p53, caspase-9, and caspase-3, ROS	In vitro	7.5 μM/0, 12, 18, and 24 h	U-2 OS	[79]
BITC	Antimetastasis	Lung	↓MMP-2, Twist, β-catenin, AKT, and NF-κB ↑ROS	In vitro	5 μM/24 h	L9981	[80]
BITC	Antitumorigenesis	Leukocyte	↓Hydrogen peroxide, ROS	In vitro In vivo	1, 10, 100 μM/24 h 81 or 810 nmol/ 20 wk	HL-60	[81]

Table 1. Continued

Compounds	Efficacy	Organ	Mechanism		Dose/duration	Cell line	References
PEITC	Apoptosis	Breast	↓HER2, EGFR, STAT3, BCL-XL, XIAP, ROS ↑Bax, Bim, HER2 ↓HER2, EGFR, STAT3	In vitro In vivo	10 μM/24 h 12 μmol/33 days	MDA-MB-231, MCF-7	[95]
PEITC	Apoptosis	Prostate	↓BCL-2, XIAP ↑Bax, Bak	In vitro In vivo	1 μM/24 h 9 μmol/38 days	PC-3, DU145	[97]
PEITC	Apoptosis	Leukemia	↑ROS, NO ↓GSH	In vitro	10 μM/1–6 h	HL-60	[98]
PEITC	Apoptosis	Breast	↑ROS, caspases 9 and 3 ↓GSH, Bax	In vitro	20 μM/6 h	MDA-MB-231, MCF-7	[99]
PEITC	Apoptosis	T-cell	↑JNK	In vitro	20 μM/24 h	Jurkat	[101]
PEITC	Apoptosis	Lung	↑JNK, p38, Erk1/2, AP-1	In vivo	15 μM/g/140 days		[102]
PEITC	Apoptosis	Colon	↓SOS-1, PKC, ERK1/2, Rho A, MMP-2 and -9, GRB2, NF-κB, iNOS, COX-2	In vitro	2.5 μM/24, 48 h	HT29	[103]
PEITC	Antimetastasis	Liver	↓MMP-2,MMP-9 ↑TIMP1, 2	In vitro	0.1–5 μM/24 h	SK-Hep1	[104]
PEITC	Antiangiogenesis	Prostate	↓VEGF, VEGF receptor 2, AKT, EGF, G-CSF	In vitro	4 μM/24 h	HUVEC, PC-3	[105]
PEITC	Antiangiogenesis	Breast	↓HIF-1α, CAIX, GLUT1, BNIP3, VEGF-A, 4E-BP1	In vitro	16 μM/24 h	MCF7	[106]
PEITC	Apoptosis, cell-cycle arrest	Prostate	↑p21WAF-1/Cip-1 and p27Kip1 ↓Cyclins D and E, Rb	In vivo	8 μmol/g/9 wk	BALB/c male mice	[122]
PEITC	Apoptosis, cell-cycle arrest	Oral squamous cell	↑p53, p21, p17, Bax, Bid ↓cyclin E, CDK2, BCL-2	In vitro	5 μM/72 h	HSC-3	[116]
PEITC	Apoptosis	Multiple myeloma	↑PARP, caspase-3 and caspase-9, c-jun, HSP27 ↓Mcl-1, X-IAP, c-IAP, and survivin	In vitro	2.5, 5, and 10 μM/12, 24, 48 h	MM.1S	[51]
PEITC	Apoptosis	Breast	↑p57 Kip2, p53, BRCA2, IL-2, and ATF-2	In vitro	3 μM/48 h	MCF-7	[124]
PEITC	Apoptosis, cell-cycle arrest	Prostate	↑p53, WEE1, caspase-3, caspase-8, caspase-9 ↓CDC25C	In vitro	20 μM/24, 48 h	DU 145	[117]
PEITC	Cell-cycle arrest	Lung	↓Cell growth	In vitro	3, 6, 9 μM/24, 48, 72 h	A549, H1299	[125]
PEITC	Apoptosis	Ovary	↓EGFR, AKT, p-GSK ↓Tumor growth	In vitro In vivo	40 μM/24 h 12 μmol/42 days	SKOV-3, OVCAR-3, TOV-21G	[109]
PEITC	Apoptosis	Oral cancer	↑Caspase-3, caspase-8, BID, DR5	In vitro	10 μM/48 h	HN22	[113]
PEITC	Apoptosis	Cervical cancer	↑DR4, DR5, caspase-3, PARP ↓ERK1/2	In vitro	5 μM/48 h	HEp-2, KB	[114]
PEITC	Apoptosis	Breast	↑Caspases 7 and 9, PARP ↓BCL-2, XIAP	In vitro	3–30 μM/24 h	MCF-7	[118]
PEITC	Apoptosis	Breast	↓ER-α36, ERK 1/2	In vitro	10 μM/48 h	MCF7, H3396, MDA-MB-231,SK-BR-3	[110]

Table 1. Continued

Compounds	Efficacy	Organ	Mechanism		Dose/duration	Cell line	References
SFN	Apoptosis	Liver	↑TrxR1	In vitro	12 μM/24 h	HepG2	[49]
SFN	Apoptosis	Breast	↑TrxR1	In vitro	3, 6 μM/24 h	MCF-7	[50]
SFN	Apoptosis	Prostate	↑Ac-histone H4, Bax, P21, ↓HDAC	In vitro	15 μM/24 h	BPH-1, LnCaP, PC-3	[133]
SFN	Apoptosis, cell-cycle arrest	Bladder	↑p27	In vitro	5–20 μM	T24	[147]
SFN	Apoptosis, cell-cycle arrest	Colon	↑Cyclins A and B1, bax, cytochrome c	In vitro	15 μm/24 h	HT29	[148]
SFN	Apoptosis, cell-cycle arrest	Lymphoblastic leukemia	↑Caspases 3, 8, and 9, p21 ↓Cdc2/Cyclin B1, AKT, mTOR	In vitro	7.5 μM/24 h	LCL, Nalm-6, Jurkat, KOPTK1	[149]
SFN	Apoptosis	Bladder	↓Tumor volume ↑Caspase 3 and cytochrome c	In vivo	12 mg/kg/5 wk	Athymic mice	[135]
SFN	Apoptosis	Prostate	↓p65, VEGF, cylcin D1, and BCL-X	In vitro	20 and 30 μM/24 h	PC-3	[136]
SFN	Cell-cycle arrest	Colon	↑ERK, JNK, p38, p21 ↓Cyclin D1	In vitro	6.25, 12.5, 25, 50, and 100 μM/24 h	HT-29	[150]
SFN	Apoptosis	Colon	↑ERK1/2 and AKT	In vitro	20, 30 μM/24 h	Caco-2	[151]
SFN	Apoptosis	Breast	↑Caspase-8, caspase-3, cytochrome c ↓BCL-2, HDAC, ERalpha, EGFR, EGFR2	In vitro	0, 5, 15, or 25 μmol/L for 48 h	MDA-MB-231, MDA-MB-468, MCF-7, T47D	[137]
SFN	Apoptosis	Prostate	↓IAP, cIAP1, cIAP2, XIAP, p65 ↑Apaf-1, E2F1	In vitro	20 μM/24 h	LNCaP, PC3	[143]
SFN	Antiangiogenesis, antimetastasis	Pancreas stem cell	↓Nanog, Oct-4, VEGF, platelet derived growth factor receptor alpha, ZEB-1	In vivo	0–20 mg/kg/6 wk	non-obese diabetic (NOD)/severe combined immunodeficiency (SCID)/IL2Rgamma mice	[144]
SFN	Apoptosis	prostate	↑ROS, Fas, caspase-8, Bid	In vitro	40 μM/4 h	PC-3	[145]
SFN	Antimetastasis	Oral carcinoma	↓MMP-1, MMP-2	In vitro	1 μM/24 h	YD8, YD10B, YD15	[152]
SFN	Apoptosis	Breast	↓Tumor growth	In vitro In vivo	25 or 50 mg/kg/24 days	KPL-1 Athymic mice	[153]
SFN	Antimetastasis	Bladder	↓ZEB-1, SNAIL ↑E-cadherin	In vitro	0–20 μM/24 h	T24	[154]
AITC	Apoptosis, cell-cycle arrest	Prostate	↓BCL-2, cyclin B1, Cdc25B, Cdc25C ↑BID	In vivo	10 μmol/26 days	Nude mice	[168]
AITC	Antiproliferation	Colon	↓Mitosis	In vitro	12 μM/24 h	HT-29	[164]
AITC	Apoptosis	Prostate	↑AP-1, ERK1/2, JNK1/2, Elk-1, and c-Jun	In vitro	50 μM/24 h	PC-3	[165]
AITC	Apoptosis, cell-cycle arrest	Bladder	↓α- and β-Tubulin ↑cytochrome c, caspase-9, caspase-3, BCL-2, JNK	In vitro	30 μM/24 h	UM-UC-3	[167]
AITC	Antimetastasis	Liver	↓MMP-2/-9	In vitro	0.1-5 μM/24 h	SK-Hep 1	[169]

ER, estrogen receptor; Rb, retinoblastoma protein.

while the other MAP kinases were playing role in the induction of apoptosis [57].

4.1.5 Invasion and metastasis

Metastasis is initiated by key regulators, such as matrix metalloproteinases, Twist, and β-catenin. A study showed that BITC treatment inhibited cell migration and invasion in lung cancer cells. This was accompanied with reduced expression of MMP-2, Twist, and β-catenin [80]. Another study showed that oral administration of 5 and 10 mg/kg BITC suppressed the expression of MMP-2 and MMP-9 in the sera and lungs of mice injected with 4T1 breast cancer cells [85]. BITC also inhibits the process of epithelial to mesenchymal transition through FOXQ1 suppression in breast cancer cells, leading to reduced metastatic potential [86]. The data available for antimetastatic effects of BITC are insufficient to prove the antimetastatic efficacy. Hence, additional elaborate studies are required to establish the role of BITC in metastasis.

4.1.6 In vivo studies

Our in vivo studies indicated that BITC is well tolerated at a dose of 12 μmol/day (72 mg/kg) in mice. Interestingly, in vivo tumor growth was markedly arrested by BITC treatment in athymic nude mice as compared to controls [59]. These results showed that after 6 wk of 12 μmol/day BITC treatment by oral gavage, average tumor volume in BITC-treated mice was about 48% less as compared to the control group [59]. LC-MS analysis showed that after 46 days of BITC (12 μmol/day) treatment, mean concentration of 6.5 ± 0.1 μmol/L (39 mg/L; $n = 10$) and 7.5 ± 0.3 μmol/g (45 mg/g; $n = 10$) BITC was observed in the plasma and tumors of treated mice, respectively [55]. These results suggest a reasonable bioavailability of BITC and also that the therapeutic concentration could be achieved in vivo by oral administration. No untoward side effect or change in body weight was observed, suggesting that 12 μmol/day BITC was relatively safe. Furthermore, suppression of in vivo angiogenesis by 12 μmol/day (72 mg/kg) treated mice was observed by reduction of hemoglobin content by 76% in matrigel plugs implanted in the mice as well by 61% in the excised tumor xenografts, as compared to respective controls [77]. These results signify the potential antitumor and antiangiogenic effects of BITC. The molecular targets of BITC have been described in detail in Table 1. Interestingly, dietary BITC also suppressed the growth of cancer stem cell in mouse mammary tumor virus-neu (MMTV-neu) breast cancer transgenic mice model along with inhibition of major stem cell markers, such as Oct4, SOX-2, and Nanog [87]. In contrast, the activation of NOTCH2 signaling by BITC was found to impede the therapeutic benefits of BITC [88]. A recent study from our group showed that the absorption and bioavailability of BITC can be enhanced by making the nanoemulsion of BITC [89].

4.1.7 Toxicity studies

No major evidence of BITC side effect exists for the doses that are commonly used for anticancer studies. A study has shown that oral administration of BITC (0, 50, 100, and 200 mg/kg) for 4 wk caused reduction in body weight and reduced food consumption only at highest doses [90]. In addition, the study revealed that BITC treatment caused increase in serum cholesterol and decrease in triglycerides, accompanied with renal dysfunction. Furthermore, in this study BITC treatment reduced the weight of almost all the organs except the adrenals, where the weight was increased. Some transitory hematological changes, such as reduced hemoglobin and lymphocyte count, with increased platelets, eosinophils, and neutrophils were observed in BITC-treated rats. It is pertinent to note that no significant signs of toxicity were observed at the dose of 50 mg/kg. Although these changes were observed at higher doses (100 and 200 mg/kg) of BITC, no mortality was reported [90]. The high doses such as 100–200 mg/kg BITC, which were associated with some side effects, are unlikely to be used for antitumor effects. So far the therapeutic doses of BITC, which suppresses in vivo tumor growth, are much lower and not associated with any side effects and hence can be considered relatively safe.

4.2 Phenethylisothiocyanate (PEITC)

PEITC is another isothiocyanate that occurs conjugated with glucosinolate in many cruciferous plants. PEITC is abundantly present in plants, such as watercress, garden cress and in some noncruciferous plants, such as turnips and radishes [30, 91]. Watercress is the most prolific source of PEITC, which can release approximately 2–6 mg PEITC/ounce (0.07–0.21 mg of PEITC/g) in humans [91, 92].

The effective concentrations of PEITC vary from 0.12 to 14 μM [93, 94]. Like BITC, PEITC also induces ROS generation selectively in cancer cells [95, 96]. Mechanistic studies have shown that PEITC disrupts mitochondrial electron transport chain by inhibiting complex I and III activity and reduces oxygen consumption rate in prostate cancer cells [97, 98]. Furthermore, PEITC is known to inhibit ROS-detoxifying mechanisms to enhance ROS-mediated cytotoxicity [96–98]. This was further proven in cells with varying levels of anti-ROS mechanisms that showed differential sensitivity toward PEITC [99, 100].

Two general mechanisms that have been identified for the anticancer activity of PEITC include cell-cycle arrest and apoptosis induction [44, 101, 102]. Few studies also suggest antiangiogenic and antimetastatic effects of PEITC by mechanisms similar to BITC [80, 103–106]. PEITC has been shown to act on about 30 different targets present in cancer cells (Fig. 3) [107]. Mi et al. have shown that PEITC alters the function of critical amino acids of proteins and peptides through covalent interactions [107].

4.2.1 Cell proliferation and growth

Studies suggest that PEITC has multiple targets, such as AKT, epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2), in cancer cells that promote antiapoptotic mechanisms in cancer cells. As discussed earlier, AKT (protein kinase B) is frequently overexpressed in cancers and regulated by oncogenes, such as EGFR and HER2 [108]. Our studies demonstrated that PEITC inhibits EGFR and HER2 in ovarian and breast cancer cells [95, 109]. PEITC caused significant inhibition of activated EGFR (Tyr1068) to suppress the growth of ovarian cancer cells. Furthermore, PEITC reduced the phosphorylation of AKT and mTOR expression [109]. In this study, PEITC also disrupted the complex of Raptor and Rictor with mTORC1 and mTORC2 [109]. In another study, we observed inhibition of HER2 and AKT in breast cancer cells. These observations suggest that PEITC inhibits AKT activation by suppressing EGFR and HER2 expressions to suppress antiapoptotic signaling in cancer cells (Table 1). Furthermore, PEITC also inhibits HDACs, the major epigenetic regulators resulting in the inhibition of androgen receptor in prostate cancer cells [40].

4.2.2 Angiogenesis

Similar to BITC, PEITC also inhibits VEGF, a major promoter of angiogenesis. Xiao and Singh showed suppression of VEGF by PEITC, which was later shown to be mediated through suppression of HIF-1α [105, 110–112]. Based on the evidence provided in these studies, it can be suggested that PEITC inhibits angiogenesis mainly by inhibiting VEGF.

4.2.3 Mitochondrial cell death

Accumulating evidence from several studies showed induction of apoptosis signaling by PEITC. PEITC has been shown to activate death receptors and Fas-mediated extrinsic apoptotic pathway in oral and cervical cancer cells [113–115]. PEITC treatment also resulted in the activation of intrinsic pathway of apoptosis. PEITC modulates mitochondrial proteins, such as BCL-2, BID, and BAX, causing the release of cytochrome c into cytosol to induce intrinsic apoptosis pathway [94, 116–119]. However, the release of cytochrome c by PEITC treatment into cytosol to induce apoptosis was contradicted by a study conducted by Wu et al. [120]. Further in-depth studies are thus required to delineate the exact mechanism of PEITC.

4.2.4 Cell-cycle arrest

PEITC as well as its *N*-acetyl cysteine conjugate causes activation of retinoblastoma protein in prostate cancer cells, leading to attenuation of cell-cycle progression [39, 121]. Furthermore, a G0/G1 phase cell-cycle arrest by PEITC was associated with activation of p53 in oral squamous carcinoma cells, in multiple myeloma, osteogenic sarcoma and breast cancer cells, and G2/M cell-cycle arrest in prostate cancer cells [51, 52, 116, 117, 122, 123]. Interestingly, lung carcinoma cells expressing mutated p53 were shown to be more sensitive to PEITC as compared to cells with wild-type p53 expression [107, 124].

4.2.5 Invasion and metastasis

PEITC inhibits cancer cell invasion by inhibiting MMPs and suppresses activity of ERK and NF-κB to inhibit metastasis [103, 104]. We recently demonstrated in vivo antimetastatic potential of PEITC using a unique mouse model of breast cancer metastasis [94]. This model utilizes MDA-MB-231-Luc2 brain-seeking breast cancer cells that lodge in the brain from blood circulation when injected into the left ventricle of mouse heart. These cells later grow to form metastatic tumors in brain. Oral administration of 10 μmol PEITC (65 mg/kg) for 10 days significantly prevented the seeding of breast cancer cells into the brain in this model. We also observed that PEITC administration suppressed the growth of metastasized tumor in the brain and enhanced the survival of mice bearing tumors in the brain [94]. This was the first evidence of in vivo antimetastatic effects of PEITC in breast cancer model, but further studies are required to establish similar efficacy in other cancer forms. The molecular targets of PEITC have been described in detail in Table 1.

4.2.6 In vivo studies

PEITC-mediated inhibition of anti-apoptotic pathways was observed in the preclinical mouse model studies [125]. PEITC has a dose-dependent bioavailability of about 70–110% by oral administration, which is a probable reason for in vivo efficacy [126]. Treatment of brain metastatic breast cancer has always been a problem due to the presence of blood–brain barrier. Organ distribution study has revealed a fair availability of PEITC in brain, suggesting better chances of PEITC to cross blood–brain barrier [127]. This could be the reason for the antimetastatic effects of PEITC [94]. These studies indicate a high antitumor efficacy of PEITC in all organs including brain by oral administration. Orally administered PEITC causes significant inhibition of major oncogenic pathways, such as EGFR, HER2, and AKT, in various in vivo cancer models leading to tumor growth suppression [94, 95, 109, 128]. These results clearly reenforce potential for in vivo efficacy of PEITC.

4.2.7 Toxicity studies

In addition to the beneficial effects, it is also essential to evaluate the probable side effects of PEITC. It was observed that intra-peritoneal (i.p.) administration of 80 and 160 mg/kg

PEITC caused increase in body weight of mice, but reduction in the weights of liver and spleen [129]. Interestingly, preventive effects of PEITC were observed on acetaminophen-induced hepatotoxicity and mortality [130]. These mutually contradicting observations make it important to establish a well-defined toxicity profile of PEITC using appropriate controls and population size.

4.2.8 Clinical studies

Three clinical studies are currently under progress to test anticancer effects in humans. A phase I lung cancer study with PEITC conducted at MD Anderson Cancer Center was recently completed; however, the findings have not yet been published. Another phase I clinical study at the same institution has been planned to test the anti-leukemic effects of PEITC. Notably, a recent phase I clinical trial (NCI CN-55120) reported that 10 μM PEITC can be achieved in the plasma after intake of 200 mg PEITC orally in human volunteers [131]. A phase II trial is also under progress in lung cancer patients at the Masonic Cancer Center, University of Minnesota in collaboration with the National Cancer Institute. The outcomes of these studies will provide data on the efficacy and toxicity of PEITC in humans.

4.3 Sulforaphane (SFN)

SFN is an isothiocyanate mainly present in broccoli and Brussels sprouts. Studies have shown that SFN is highly effective in blocking carcinogenesis (Fig. 3). SFN inhibits HDAC activity to promote cell-cycle arrest and apoptosis in $Nrf2^{-/-}$ cells suggesting Nrf2 independent mechanism of SFN [132, 133].

4.3.1 Cell proliferation and growth

SFN acts on certain molecular targets, such as survivin and NF-κB, that are vital for cancer cell survival [134, 135]. SFN induces apoptosis in breast cancer cells by the inhibition of estrogen receptor, EGFR1, and HER2, which are particularly important for the growth of breast cancer [136]. Recently SFN was shown to cause DNA damage through enhanced acetylation of DNA repair proteins. This effect was shown to be specific for cancer cells as there were no epigenetic changes or DNA damage observed in noncancer cells [137]. Interestingly, based on the methylation of DNA and cyclin D2 by SFN, a clinical trial (NCT01265953) has also been initiated at Portland, VA Medical Center [138]. Studies suggest significant epigenetic changes induced by SFN in various cancer models.

4.3.2 Angiogenesis

Very few studies have reported the antiangiogenic effects of SFN. The suppression of VEGF and MMP-2 has been shown by SFN treatment [139, 140]. Another study indicated that VEGF suppression was mediated through inhibition of FOXO1/AKT pathway [141]. However, no further evidence exists for the antiangiogenic effects of SFN. Due to the lack of sufficient evidence, antiangiogenic activity cannot be considered as a critical mechanism of SFN.

4.3.3 Mitochondrial cell death

Another important mechanism of action of SFN was inactivation of inhibitors of apoptosis proteins [142]. SFN-mediated inhibitors of apoptosis protein inhibition was associated with BCL-2 inhibition, suggesting activation of intrinsic apoptosis pathway [143]. SFN also causes generation of mitochondrial ROS in cancer cells that further leads to release of cytochrome c into cytosol augmenting cell apoptosis [144]. Interestingly, an ROS independent activation of MEK/ERK pathway was shown to lead to caspase-dependent apoptosis in neuroblastoma cells [145].

4.3.4 Cell-cycle arrest

SFN was shown to induce p27-mediated G0/G1 phase cell-cycle arrest [146]. In addition, SFN causes irreversible cell-cycle arrest in G2/M phase followed by caspase-mediated apoptosis [147]. Recent studies have shown that SFN induces G2/M arrest through the activation of p21 (CIP1/WAF1) and inhibition of Cdc2/Cyclin B1 complex independent of p53 [148]. This study showed that apoptosis following G2/M arrest was induced by caspase and PARP activation in leukemia cells [148]. Specific activation of MAP kinases, such as ERK, JNK, and p38, in response to SFN treatment was shown to be involved in inducing cell-cycle arrest [149, 150].

4.3.5 Invasion and metastasis

SFN exhibits potent antimetastatic effects by suppressing cell migration and invasion. Jee et al. observed that the anti-cell migratory effect of SFN was associated with MMP suppression [151]. Recently epithelial to mesenchymal transition (EMT) was shown to be an important mechanism of SFN to inhibit cell migration and metastasis in different cancer types [143, 151–153]. Li et al. have shown that SFN modulates Sonic hedgehog pathway to suppress self-renewal capacity of the pancreatic cancer stem cells and reduce EMT characteristics [143]. Significant suppression of SNAIL and ZEB-1 marked by the reexpression of E-cadherin was observed by SFN treatment that lead to reversal of EMT [153]. EMT prevention by SFN was also associated with induction of miR-200c and reexpression of the estrogen receptor [154]. The details of molecular targets of SFN have been described in Table 1.

4.3.6 In vivo studies

Kanematsu et al. demonstrated the in vivo efficacy of SFN against tumor growth and metastasis in breast cancer [152]. Pharmacokinetic studies show good bioavailability of SFN after oral administration. A concentration of 20 μM in plasma was achieved after oral administration of 50 μmol SFN/rat (35 mg/kg) [155]. In a human study it was shown that after consumption of 200 μmol SFN (35.5 mg), about 2 pmol/mg (0.355 ng/mg) SFN was detected in the breast tissue, suggesting its availability at the tumor site [156]. The cumulative concentration of SFN in the small intestine was shown to be sufficient to inhibit tumor growth in the colonic tissue [157]. These studies clearly indicate bioavailability and favorable pharmacokinetic profile of SFN that can be instrumental for future development of SFN as an anticancer agent.

4.3.7 Toxicity studies

Along with the anticancer activity of SFN, it is important to study its toxicity to assess to benefit to risk ratio. An increase in hepatoxicity indicators aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) in plasma was observed with SFN (1.6 mg/mouse/day [64 mg/kg] for 14 wk) administration in mouse bearing benzo(a)pyrene-induced (B(a)P; 100 mg/kg b.wt.) lung cancer [158]. Interestingly, opposite findings were reported in another study. The rats were pretreated with 3 mg/kg SFN by intraperitoneal injection. One hour later an intestinal ischemia/reperfusion surgery was performed to induce toxicity. It was observed that SFN administration increased the SOD levels along with reduction of myeloperoxidase, ALT, and AST levels in serum [159]. Both the studies used significantly different concentrations of SFN, which can explain the opposite observations. However, due to the lack of confirmatory evidence, overall no conclusion can be drawn about the toxicity of SFN.

4.4 Allyl isothiocyanate (AITC)

AITC is an aliphatic isothiocyanate derived from sinigrin and is excreted as NAC conjugates in the urine [160]. A recent study demonstrated a short-term reversible DNA damage when AITC was provided in the diet [161]. Cancer cells in general are more susceptible to DNA damage leading to cell death. This explains the enhanced sensitivity of cancer cells toward AITC. The cytotoxic effects of AITC were shown to be specific to cancer cells (Fig. 3) [162]. Smith et al. demonstrated apoptosis induction by AITC in colorectal cancer cells [163].

4.4.1 Cell Proliferation and growth

AITC targets specific signaling molecules to suppress cancer cell growth. ERK and JNK signaling were involved in the activation of AP-1 by AITC to suppress cancer cell growth [164, 165].

4.4.2 Mitochondrial cell death

Geng et al. observed that AITC resulted in the phosphorylation of BCL-2 to induce apoptosis, whereas mutated BCL-2 abrogated the cytotoxic effects of AITC [166].

4.4.3 Cell-cycle arrest

Srivastava et al. demonstrated the in vivo efficacy of AITC in prostate cancer [167]. This study indicated that cell growth arrest in G2/M phase by AITC was associated with the inhibition of cyclin B1, cell division cycle (Cdc)25B and Cdc25C.

4.4.4 Invasion and metastasis

The antimetastatic effects of AITC have been demonstrated through suppression of cell migration and invasion. It was observed that AITC inhibits MMP2/9 to exhibit antimetastatic effects in hepatoma cells [168]. Furthermore, AITC exerts antiangiogenic effects to suppress tumor growth by downregulating angiogenic factors, such as nitric oxide and tumor necrosis factor α (TNF-α; Table 1).

4.4.5 In vivo studies

AITC was shown to inhibit tumor and ascites formation from Ehrlich ascites tumor cells in mice. This study also revealed enhanced survival of ascites-bearing mice with AITC treatment [169]. Furthermore, i.p. administration of 25 μg AITC/animal (1 mg/kg) in mice inhibited tumor-directed capillary formation, suggesting inhibition of angiogenesis. AITC treatment also reduced serum nitric oxide and TNF-α levels indicating reduction in inflammatory markers by AITC [170]. These studies suggest a good in vivo efficacy of AITC. Nonetheless, more studies are required to confirm the in vivo activity against contemporary targets in cancer.

4.4.6 Toxicity studies

Preclinical studies have demonstrated some toxicity induced by AITC. Significant hematological changes were observed with AITC treatment. Subcutaneous administration of 20 mg/kg AITC reduced WBC counts by 25% along with marked reduction of lymphocytes and monocytes. In addition, increase in neutrophil and corticosteroid levels were observed, indicating stress induced by AITC. The AITC treatment caused reduction in thymus weights while increasing the weights of adrenals [171]. These observations suggest

significant effect of AITC on blood profile and organ weights. Interestingly in another study, i.p. administration of 25 μg AITC/animal every day for five consecutive days showed reduced WBC count at the 9th day after starting the treatment [172]. Perhaps the differences between these observations could be due to different doses and the time points of analysis after AITC administration. Another study showed increased AST levels at high doses of AITC (100–150 mg/kg), but no change was observed at lower dose (50 mg/kg), suggesting dose-dependent toxicity induced by AITC [173]. Interestingly, oral administration of AITC resulted in bladder toxicity in rats. This was found due to free AITC cleaved from urinary metabolites [174]. Taken together, studies suggest that AITC exhibits toxic side effects, cautioning its use. Further in-depth studies are required to establish the toxicity profile of AITC so that the dose for anticancer effects can be titrated effectively.

5 Potential for combination therapy

Cancer cells contain multiple aberrant signaling pathways that lead to drug resistance and therapy failure in many patients. Combination therapy is known to kill cancer cells more effectively through diverse mechanisms simultaneously. ITCs exhibit a diverse range of cellular targets for anticancer effect. This property of ITCs makes them highly desirable for combinatorial therapeutic approaches. Several combination strategies have been tested in preclinical studies by combining ITCs among themselves or with conventional or new anticancer therapies (Table 2) [58, 175–182].

Radiation therapy is an important intervention for majority of cancers. Radiation has been shown to activate some important cancer cell survival signaling molecules, such as AKT, ERK, and MCL-1 that lead to reduced efficacy. Our studies have shown that when BITC was combined with radiation therapy, a 2.8-fold increase in apoptosis and cleavage of caspase-3 was achieved in pancreatic cancer cells [58]. In addition to increased apoptosis, inhibition of NF-κB and activation of p38 was also observed with the combination of BITC and radiation therapy [58]. The combination of BITC or SFN with the radiation therapy caused increased G2/M cell-cycle arrest [58, 175]. Combination of SFN with radiation therapy also showed inhibition of activation of critical molecules, such as AKT, ERK, and MCL-1, along with induction of ER stress, explaining its efficacy [175, 183–187].

TNF-related apoptosis-inducing ligand (TRAIL) is a potential chemotherapeutic agent. Interestingly, TRAIL death receptors are highly expressed on cancer cells but not on normal cells making the cancer cells more susceptible to TRAIL-induced apoptosis as compared to normal cells [188, 189]. However, resistance to TRAIL is reported in many cancer cells [189, 190]. Our studies showed that BITC sensitized pancreatic cancer cells to TRAIL-induced apoptosis by activating both intrinsic and extrinsic pathway [191].

Accumulating evidence shows that combination of ITCs with conventional chemotherapeutics improves the efficacy against resistant cancer cells. Studies suggest synergistic activity of ITCs with common anticancer agents, such as cisplatin, adriamycin, etoposide, paclitaxel, metformin, vorinostat, and docetaxel [176, 181, 192–194]. Both BITC and PEITC increased the apoptotic effects of cisplatin through depletion of β-tubulin, but the combination did not affect DNA platination [180, 192]. Furthermore, reversal of the resistance to cisplatin was observed with PEITC, which was mediated by depletion of cellular GSH [176]. The combination of metformin and PEITC also showed high efficacy in cisplatin resistant cancer cells [177]. PEITC and SFN caused inhibition of antiapoptotic proteins, such as protein kinase C (α, β, ε, and ς), and telomerase, while increasing proapoptotic protein kinase Cδ to enhance the apoptosis caused by adriamycin and etoposide [194]. Also, the combination of adriamycin with SFN induced sensitivity in resistant cancer cells by the effect of adriamycin independent of p53 [193]. An HDAC inhibitor, vorinostat, induced ROS to increase resistance in cancer cells. PEITC treatment suppressed the cytoprotective antioxidant response through depletion of cellular ROS to reverse the resistance in leukemia cells [178]. The efficacy of taxanes was also enhanced by PEITC in different forms of cancer [97, 195]. The combination of SFN with oxaliplatin caused increased DNA fragmentation, suggesting synergism through oxaliplatin dependent mechanism [196]. NF-κB is a known target of SFN [197]. NF-κB inhibition by SFN mediated synergism with sorafenib and 5-fluorouracil to inhibit pancreatic cancer stem cell survival and salivary gland adenoid cystic carcinoma, respectively [179, 181]. These observations suggest that ITCs can utilize the mechanisms of action of conventional agents or can induce independent effects to exhibit synergism.

Although most of the combinations exhibited synergistic effects in cancer cells, a combination of 5-fluorouracil with SFN showed antagonistic activity in the normal cells by modulating G2/M cell-cycle phase [198]. This suggests that ITCs protect normal cells from the toxic effects of conventional therapeutic agents. Another study showed that the combination of cisplatin with ITCs was selectively effective in cancer cells [180]. Although the mechanism of selectivity remains to be elucidated, these observations clearly suggest an urgent need for clinical testing of the combination therapies of ITCs with conventional anticancer chemotherapeutics.

ITCs have been shown to offer synergism among themselves and other anticancer compounds. 3,3′-diindolylmethane is an important constituent of cruciferous vegetables and exhibits anticancer effects [199]. 3,3′-diindolylmethane synergizes with SFN leading to enhanced cell-cycle arrest in colon cancer cells [200]. Among other ITCs, combination of BITC with SFN or PEITC was more effective in preventing pancreatic and lung cancer than the individual treatment [201, 202]. Curcumin is a well-known dietary agent with remarkable anticancer activity [203, 204]. The combination of curcumin with ITCs caused significant reduction in

Table 2. Effects of combinations of ITC with other anticancer agents

ITCs	Concentrations and treatment time of ITCs	Combination with other agents	Concentrations	Cancer type	Targets affected by combination treatment	Effect of combined treatment	References
BITC	5 μM (18 h)	TRAIL	10 ng/mL (6 h)	Pancreatic	↑Caspase cleavage, ↓XIAP, ↑ BID cleavage, ↑ PARP cleavage	Synergistic	[192]
	20 μM (1 h)	Cisplatin	15, 30, 45 μM (48 h)	Lung	↓ β-Tubulin	Sensitization	[193]
	10 μM (3 days)	SFN	10 μM (3 days)	Pancreatic	↓ STAT3	Synergistic	[202]
	9, 12 μmol (10 wk)	PEITC (10 wk)	12 μmol	Lung	↓ Chemically induced tumorigenesis	Synergistic	[203]
	2.5 μM (24 h)	Radiation	5 Gy (24–48 h)	Pancreatic	↑ Caspase cleavage, ↑ G2/M cell-cycle arrest, ↑ ATR, ↑ Chk2, ↑ Cdc25c, ↑ Cdk-1, ↑ p21Waf1/Cip1	Synergistic	[58]
PEITC	5, 10 μM (8, 24 h)	Cisplatin	5, 10 μM (8, 24 h)	Cervical	↑ ERK, ↑ JNK, ↑ p38, ↑ MAPK	Synergistic	[181]
	20 μM (1 h)	Cisplatin	15, 30, 45 μM (48 h)	Lung	↓ β-Tubulin	Sensitization	[193]
	0.1, 0.5, 2.5, 5 μM (24 h)	Adriamycin	20, 25 μM (24 h)	Cervical	↓ PKC, ↓ telomerase	Synergistic	[195]
	0.1, 0.5, 2.5, 5 μM (24 h)	Etoposide	20, 25 μM (24 h)	Cervical	↓ PKC, ↓ telomerase	Synergistic	[195]
	5 μM (48 h)	Paclitaxel	10 nM (48 h)	Breast	↑ G2/M cell-cycle arrest	Synergistic	[196]
	5 μM (24 h), 25 mg/kg (50 days)	Platinum agents	40 μM (24 h), 5 mg/kg (50 days)	Lung	↓ GSH-mediated export, ↑ ROS, ↑ DNA damage	Sensitization	[177]
	5 μM (24 h)	Metformin	8 mM (24 h)	Ovarian (cisplatin resistant)	↑ ROS	Synergistic	[178]
	2.5 μM (6 h)	Vorinostat	2 μM (18 h)	Leukemia	↑ ROS	Synergistic	[179]
	2 μM (24 h)	Docetaxel	1nM (24 h)	Prostate	↑ XIAP, ↓ BCL-2	Synergistic	[97]
	0.025% in diet (10 and 16 wk)	Curcumin	1% in diet (10 and 16 wk)	Prostate	↓ PDK-1/AKT	Synergistic	[208]
	2.5 μmol (28 days)	Curcumin	3 μmol (28 days)	Prostate	↓ AKT	Synergistic	[209]
	10 μM (24 h)	Curcumin	25 μM (24 h)	Prostate	↓ EGFR, ↓ AKT, ↓ NF-κB	Synergistic	[210]
	2 μM (24 h)	SFN	0.4 μM (24 h)	Leukemia	↓ iNOS, ↓ COX-2, ↓ prostaglandin E2, ↓ tumor necrosis factor (TNF), ↓ IL-1	Synergistic	[206]
	0.008% in diet (17 wk)	d-Limonene, indole-3 carbinol	0.63%, 0.18% in diet (17 wk)	Lung	↓ Chemically induced tumorigenesis	Synergistic	[183]

Table 2. Continued

ITCs	Concentrations and treatment time of ITCs	Combination with other agents	Concentrations	Cancer type	Targets affected by combination treatment	Effect of combined treatment	References
SFN	20 μM (24, 48 h)	Radiation	2 Gy (24, 48 h)	Osteosarcoma	↑ Caspase cleavage, ↓ ERK, ↓ AKT, ↓ G2/M cell-cycle arrest	Synergistic	[176]
	10 μM (0, 12, 24, 48, 72 h)	Radiation	4 Gy (0, 12, 24, 48, 72 h)	Head and neck cancer	↓ AKT, ↓ MCL-1	Synergistic	[184]
	3 μM (24 h)	Arsenic trioxide	1 μM (24 h)	Multiple myeloma	↑ ER stress, ↑ HSP90, ↑ PERK, ↑ eIF2α, ↑ unfolded protein response, ↑ ROS	Synergistic	[185]
	20–30 μM (16 h)	5-FU	45–60 μM (16 h)	Salivary gland adenoid cystic carcinoma	↓ NF-κB	Synergistic	[182]
	25 μM (24 h)	Adriamycin	0.6–10 μM (24 h)	Adriamycin resistant mouse fibroblasts with mutant p53	↑p53	Sensitization	[194]
	1–20 μM (6, 24 h)	Oxaliplatin	100 nM–10 μM (6, 24 h)	Colorectal cancer	↑ DNA fragmentation	Synergistic	[197]
	10 μM (72 h) 3 mg/kg (3 days)	Sorafenib	20 μM (48 h) 60 mg/kg (3 days)	Pancreatic cancer stem cells	↓ ALDH1, ↓ NF-κB	Synergistic	[180]
	25 μM (24 h)	Resveratrol	25 μM (24 h)	Glioma cells	↓ LDH, ↓ AKT, ↑ caspase-3 cleavage	Synergistic	[186]
	20 μM (48, 72 h)	Diindolylmethane	20 μM (48, 72 h)	Colon	↑ G2/M cell-cycle arrest	Antagonistic (at low concentrations) Synergistic (at higher concentrations)	[201]
		Quercetin		Pancreatic cancer stem cells	↓ BCL-2, ↓ XIAP, ↑ caspase 3 cleavage	Synergistic	[187]
	10, 20 μM (16 h)	Quercetin	25, 50 μM (16 h)	Melanoma	↓ MMP-9	Synergistic	[188]
	10 μM (6 days)	EGG	20 μM (6 days)	Ovarian	↓ hTERT, ↓ BCL-2	Synergistic	[213]
	25 μM (24 h) 45 mg/kg (3, 12 h)	EGG	20, 100 μM (24 h) 100 mg/kg (3, 12 h)	Prostate	↓ Nrf2, ↓ AP-1	Synergistic	[212]

ER, estrogen receptor; MAPK, mitogen-activated protein kinase.

the levels of inflammatory markers. These observations advocate the possible synergistic or additive effect of curcumin in combination with ITCs [205, 206]. Several other studies reenforce the enhanced anticancer effect of PEITC with curcumin through inhibition of prosurvival pathways, such AKT, EGFR, and NF-κB [207–209]. Epigallocatechin gallate (EGG), a green tea agent, has significant anticancer potential [210]. The chemopreventive effects of the combination of SFN with EGG were successfully shown in transgenic model of prostate cancer through the induction of Nrf2 and AP-1 in Nrf2-deficient mice [211]. Furthermore, the combined treatment of SFN with EGG enhanced apoptosis in paclitaxel-resistant cancer cells by inhibiting hTERT and BCL-2 expression, showing therapeutic anticancer potential [212]. Taken together, it is clear from the above the studies that ITCs can be used for combination therapeutics in cancer treatment, especially for the resistant cancers. The combinations of ITCs with various anticancer agents and their prime mechanism of action have been summarized in Table 2.

6 Conclusion

Current epidemiological studies have certain limitations, such as differential exposure of the populations leading to misclassification, improper controls, and possibility of recall bias. Hence, better designed studies are required to establish the role of ITCs as neutraceuticals for cancer prevention and treatment. Furthermore, better designed studies along with detailed mechanistic studies can provide us with an opportunity to use ITCs as the lead for synthesis of more potent and safe drugs through chemical modifications. It is important to note that some studies were done using extracts of ITCs from the vegetables. Few studies have shown that ITCs are susceptible to hydrolytic degradation at high temperatures and basic conditions [213, 214]. Thus, the observations made by extracts of ITCs could be questionable especially if the extraction procedure was not appropriate or standard. These observations require further confirmation using pure forms of ITCs.

Recent studies have revealed many novel cancer targets. Specifically, targeting these can enhance the efficacy of new as well as conventional therapies. Hence, it is important to test the efficacy of ITCs against new targets. Current preclinical evidence presented in the review provides an insight into potential anticancer mechanisms of action of the ITCs as well as their selectivity toward the cancer cells. Some clinical studies have been initiated already for some ITCs. Nonetheless, further detailed studies are required to establish the safety and efficacy profiles of these agents based on which they can be streamlined for further human studies. Based on the current data, it is evident that ITCs possess highly potential anticancer activity, but further detailed toxicity and clinical studies are required to warrant their future clinical benefits.

It should be noted that although we tried to include most of the published papers related to the objective of this review, it is possible that by mistake we may have missed a few papers. For this we would like to apologize to those authors.

In order to maintain consistency, we converted all the doses of ITCs into metric units, which initially appeared in different forms in the literature. The conversion was made assuming the average weight of mice as 25 g and average weight of rats as 250 g. We would like to apologize for any deviation that might have occurred during unit conversion from the dose used in the actual study. We therefore included the units reported by the authors as well as values converted into metric units by us in parenthesis.

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7 References

[1] Newman, D. J., Cragg, G. M., Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 2012, *75*, 311–335.

[2] Higdon, J. V., Delage, B., Williams, D. E., Dashwood, R. H., Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol. Res.* 2007, *55*, 224–236.

[3] Boggs, D. A., Palmer, J. R., Wise, L. A., Spiegelman, D. et al., Fruit and vegetable intake in relation to risk of breast cancer in the Black Women's Health Study. *Am. J. Epidemiol.* 2010, *172*, 1268–1279.

[4] Block, G., Patterson, B., Subar, A., Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 1992, *18*, 1–29.

[5] Tang, L., Zirpoli, G. R., Guru, K., Moysich, K. B. et al., Intake of cruciferous vegetables modifies bladder cancer survival. *Cancer Epidemiol. Biomarkers Prev.* 2010, *19*, 1806–1811.

[6] Palmer, S., Diet, nutrition, and cancer. *Prog. Food Nutr. Sci.* 1985, *9*, 283–341.

[7] Boreddy, S. R., Srivastava, S. K., Pancreatic cancer chemoprevention by phytochemicals. *Cancer Lett.* 2012, 86–94.

[8] Fowke, J. H., Chung, F. L., Jin, F., Qi, D. et al., Urinary isothiocyanate levels, Brassica, and human breast cancer. *Cancer Res.* 2003, *63*, 3980–3986.

[9] Ambrosone, C. B., McCann, S. E., Freudenheim, J. L., Marshall, J. R. et al., Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. *J. Nutr.* 2004, *134*, 1134–1138.

[10] Bosetti, C., Negri, E., Franceschi, S., Pelucchi, C. et al., Diet and ovarian cancer risk: a case-control study in Italy. *Int. J. Cancer* 2001, *93*, 911–915.

[11] Zhang, M., Yang, Z. Y., Binns, C. W., Lee, A. H., Diet and ovarian cancer risk: a case-control study in China. *Br J. Cancer* 2002, *86*, 712–717.

[12] Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K. et al., Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 501–508.

[13] Stoner, G. D., Morse, M. A., Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett.* 1997, *114*, 113–119.

[14] Zhang, Y., Talalay, P., Cho, C. G., Posner, G. H., A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA* 1992, *89*, 2399–2403.

[15] Fimognari, C., Lenzi, M., Hrelia, P., Interaction of the isothiocyanate sulforaphane with drug disposition and metabolism: pharmacological and toxicological implications. *Curr. Drug Metab.* 2008, *9*, 668–678.

[16] Gross-Steinmeyer, K., Stapleton, P. L., Liu, F., Tracy, J. H. et al., Phytochemical-induced changes in gene expression of carcinogen-metabolizing enzymes in cultured human primary hepatocytes. *Xenobiotica* 2004, *34*, 619–632.

[17] Nakajima, M., Yoshida, R., Shimada, N., Yamazaki, H. et al., Inhibition and inactivation of human cytochrome P450 isoforms by phenethyl isothiocyanate. *Drug Metab. Dispos.* 2001, *29*, 1110–1113.

[18] Yoshigae, Y., Sridar, C., Kent, U. M., Hollenberg, P. F., The inactivation of human CYP2E1 by phenethyl isothiocyanate, a naturally occurring chemopreventive agent, and its oxidative bioactivation. *Drug Metab. Dispos.* 2013, *41*, 858–869.

[19] Morse, M. A., Amin, S. G., Hecht, S. S., Chung, F. L., Effects of aromatic isothiocyanates on tumorigenicity, O6-methylguanine formation, and metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Cancer Res.* 1989, *49*, 2894–2897.

[20] Wattenberg, L. W., Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J. Natl. Cancer Inst.* 1977, *58*, 395–398.

[21] Adam-Rodwell, G., Morse, M. A., Stoner, G. D., The effects of phenethyl isothiocyanate on benzo[a]pyrene-induced tumors and DNA adducts in A/J mouse lung. *Cancer Lett.* 1993, *71*, 35–42.

[22] Conaway, C. C., Wang, C. X., Pittman, B., Yang, Y. M. et al., Phenethyl isothiocyanate and sulforaphane and their N-acetylcysteine conjugates inhibit malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice. *Cancer Res.* 2005, *65*, 8548–8557.

[23] Hecht, S. S., Approaches to chemoprevention of lung cancer based on carcinogens in tobacco smoke. *Environ. Health Perspect.* 1997, *105* (Suppl 4), 955–963.

[24] Morse, M. A., Wang, C. X., Amin, S. G., Hecht, S. S. et al., Effects of dietary sinigrin or indole-3-carbinol on O6-methylguanine-DNA-transmethylase activity and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumorigenicity in F344 rats. *Carcinogenesis* 1988, *9*, 1891–1895.

[25] Munday, R., Munday, C. M., Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds. *J. Agric. Food Chem.* 2004, *52*, 1867–1871.

[26] Jiao, D., Conaway, C. C., Wang, M. H., Yang, C. S. et al., Inhibition of N-nitrosodimethylamine demethylase in rat and human liver microsomes by isothiocyanates and their glutathione, L-cysteine, and N-acetyl-L-cysteine conjugates. *Chem. Res. Toxicol.* 1996, *9*, 932–938.

[27] Moreno, R. L., Kent, U. M., Hodge, K., Hollenberg, P. F., Inactivation of cytochrome P450 2E1 by benzyl isothiocyanate. *Chem. Res. Toxicol.* 1999, *12*, 582–587.

[28] Steinkellner, H., Rabot, S., Freywald, C., Nobis, E. et al., Effects of cruciferous vegetables and their constituents on drug metabolizing enzymes involved in the bioactivation of DNA-reactive dietary carcinogens. *Mutat. Res.* 2001, 480–481, 285–297.

[29] Zhou, C., Poulton, E. J., Grun, F., Bammler, T. K. et al., The dietary isothiocyanate sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor. *Mol. Pharmacol.* 2007, *71*, 220–229.

[30] Cheung, K. L., Kong, A. N., Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *AAPS J.* 2010, *12*, 87–97.

[31] Brooks, J. D., Paton, V. G., Vidanes, G., Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 949–954.

[32] Talalay, P., Fahey, J. W., Holtzclaw, W. D., Prestera, T. et al., Chemoprotection against cancer by phase 2 enzyme induction. *Toxicol. Lett.* 1995, *82–83*, 173–179.

[33] Zhang, Y., Talalay, P., Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res.* 1994, *54*, 1976s–1981s.

[34] Hecht, S. S., Inhibition of carcinogenesis by isothiocyanates. *Drug Metab. Rev.* 2000, *32*, 395–411.

[35] Mannervik, B., The isoenzymes of glutathione transferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* 1985, *57*, 357–417.

[36] Hayes, J. D., Pulford, D. J., The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* 1995, *30*, 445–600.

[37] Thornalley, P. J., Isothiocyanates: mechanism of cancer chemopreventive action. *Anticancer Drugs* 2002, *13*, 331–338.

[38] Zhang, Y., Role of glutathione in the accumulation of anticarcinogenic isothiocyanates and their glutathione conjugates by murine hepatoma cells. *Carcinogenesis* 2000, *21*, 1175–1182.

[39] Beklemisheva, A. A., Feng, J., Yeh, Y. A., Wang, L. G. et al., Modulating testosterone stimulated prostate growth by phenethyl isothiocyanate via Sp1 and androgen receptor down-regulation. *Prostate* 2007, *67*, 863–870.

[40] Wang, L. G., Chiao, J. W., Prostate cancer chemopreventive activity of phenethyl isothiocyanate through epigenetic regulation (review). *Int. J. Oncol.* 2010, *37*, 533–539.

[41] Kong, A. N., Yu, R., Hebbar, V., Chen, C. et al., Signal transduction events elicited by cancer prevention compounds. *Mutat. Res.* 2001, *480–481*, 231–241.

[42] Keum, Y. S., Yu, S., Chang, P. P., Yuan, X. et al., Mechanism of action of sulforaphane: inhibition of p38 mitogen-activated protein kinase isoforms contributing to the induction of antioxidant response element-mediated heme oxygenase-1 in human hepatoma HepG2 cells. *Cancer Res.* 2006, *66*, 8804–8813.

[43] Prawan, A., Keum, Y. S., Khor, T. O., Yu, S. et al., Structural influence of isothiocyanates on the antioxidant response element (ARE)-mediated heme oxygenase-1 (HO-1) expression. *Pharm. Res.* 2008, *25*, 836–844.

[44] Keum, Y. S., Jeong, W. S., Kong, A. N., Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. *Mutat. Res.* 2004, *555*, 191–202.

[45] Zhang, C., Su, Z. Y., Khor, T. O., Shu, L. et al., Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. *Biochem. Pharmacol.* 2013, *85*, 1398–1404.

[46] Fimognari, C., Hrelia, P., Sulforaphane as a promising molecule for fighting cancer. *Mutat. Res.* 2007, *635*, 90–104.

[47] Brigelius-Flohe, R., Banning, A., Part of the series: from dietary antioxidants to regulators in cellular signaling and gene regulation. Sulforaphane and selenium, partners in adaptive response and prevention of cancer. *Free Radic. Res.* 2006, *40*, 775–787.

[48] Bacon, J. R., Plumb, G. W., Howie, A. F., Beckett, G. J. et al., Dual action of sulforaphane in the regulation of thioredoxin reductase and thioredoxin in human HepG2 and Caco-2 cells. *J. Agric. Food Chem.* 2007, *55*, 1170–1176.

[49] Zhang, J., Svehlikova, V., Bao, Y., Howie, A. F. et al., Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. *Carcinogenesis* 2003, *24*, 497–503.

[50] Wang, W., Wang, S., Howie, A. F., Beckett, G. J. et al., Sulforaphane, erucin, and iberin up-regulate thioredoxin reductase 1 expression in human MCF-7 cells. *J. Agric. Food Chem.* 2005, *53*, 1417–1421.

[51] Jakubikova, J., Cervi, D., Ooi, M., Kim, K. et al., Antitumor activity and signaling events triggered by the isothiocyanates, sulforaphane and phenethyl isothiocyanate, in multiple myeloma. *Haematologica* 2011, *96*, 1170–1179.

[52] Huang, C., Ma, W. Y., Li, J., Hecht, S. S. et al., Essential role of p53 in phenethyl isothiocyanate-induced apoptosis. *Cancer Res.* 1998, *58*, 4102–4106.

[53] Yu, R., Mandlekar, S., Harvey, K. J., Ucker, D. S. et al., Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. *Cancer Res.* 1998, *58*, 402–408.

[54] Zhang, Y., Callaway, E. C., High cellular accumulation of sulphoraphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. *Biochem. J.* 2002, *364*, 301–307.

[55] Boreddy, S. R., Pramanik, K. C., Srivastava, S. K., Pancreatic tumor suppression by benzyl isothiocyanate is associated with inhibition of PI3K/AKT/FOXO pathway. *Clin. Cancer Res.* 2011, *17*, 1784–1795.

[56] Batra, S., Sahu, R. P., Kandala, P. K., Srivastava, S. K., Benzyl isothiocyanate-mediated inhibition of histone deacetylase leads to NF-kappaB turnoff in human pancreatic carcinoma cells. *Mol. Cancer Ther.* 2010, *9*, 1596–1608.

[57] Sahu, R. P., Zhang, R., Batra, S., Shi, Y. et al., Benzyl isothiocyanate-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of MAPK in human pancreatic cancer cells. *Carcinogenesis* 2009, *30*, 1744–1753.

[58] Sahu, R. P., Epperly, M. W., Srivastava, S. K., Benzyl isothiocyanate sensitizes human pancreatic cancer cells to radiation therapy. *Front. Biosci.* 2009, *1*, 568–576.

[59] Sahu, R. P., Srivastava, S. K., The role of STAT-3 in the induction of apoptosis in pancreatic cancer cells by benzyl isothiocyanate. *J. Natl. Cancer Inst.* 2009, *101*, 176–193.

[60] Zhang, R., Loganathan, S., Humphreys, I., Srivastava, S. K., Benzyl isothiocyanate-induced DNA damage causes G2/M cell cycle arrest and apoptosis in human pancreatic cancer cells. *J. Nutr.* 2006, *136*, 2728–2734.

[61] Srivastava, S. K., Singh, S. V., Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis* 2004, *25*, 1701–1709.

[62] Kalkunte, S., Swamy, N., Dizon, D. S., Brard, L., Benzyl isothiocyanate (BITC) induces apoptosis in ovarian cancer cells in vitro. *J. Exp. Ther. Oncol.* 2006, *5*, 287–300.

[63] Nakamura, Y., Kawakami, M., Yoshihiro, A., Miyoshi, N. et al., Involvement of the mitochondrial death pathway in chemopreventive benzyl isothiocyanate-induced apoptosis. *J. Biol. Chem.* 2002, *277*, 8492–8499.

[64] Finkielsztein, A., Kelly, G. M., Altering PI3K-Akt signalling in zebrafish embryos affects PTEN phosphorylation and gastrulation. *Biol. Cell* 2009, *101*, 661–678.

[65] Schlieman, M. G., Fahy, B. N., Ramsamooj, R., Beckett, L. et al., Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br. J. Cancer* 2003, *89*, 2110–2115.

[66] Sarbassov, D. D., Guertin, D. A., Ali, S. M., Sabatini, D. M., Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005, *307*, 1098–1101.

[67] Lin, J. F., Tsai, T. F., Liao, P. C., Lin, Y. H. et al., Benzyl isothiocyanate induces protective autophagy in human prostate cancer cells via inhibition of mTOR signaling. *Carcinogenesis* 2013, *34*, 406–414.

[68] Xiao, D., Bommareddy, A., Kim, S. H., Sehrawat, A. et al., Benzyl isothiocyanate causes FoxO1-mediated autophagic death in human breast cancer cells. *PLoS One* 2012, *7*, e32597.

[69] Ghosh, S., May, M. J., Kopp, E. B., NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 1998, *16*, 225–260.

[70] Sun, Z., Andersson, R., NF-kappaB activation and inhibition: a review. *Shock* 2002, *18*, 99–106.

[71] Darnell, J. E., Validating Stat3 in cancer therapy. *Nat. Med.* 2005, *11*, 595–596.

[72] Wei, D., Le, X., Zheng, L., Wang, L. et al., Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene* 2003, *22*, 319–329.

[73] Fouse, S. D., Costello, J. F., Cancer stem cells activate STAT3 the EZ way. *Cancer Cell* 2013, *23*, 711–713.

[74] Semenza, G. L., HIF-1 and mechanisms of hypoxia sensing. *Curr. Opin. Cell Biol.* 2001, *13*, 167–171.

[75] Xu, Q., Briggs, J., Park, S., Niu, G. et al., Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene* 2005, *24*, 5552–5560.

[76] Jung, J. E., Lee, H. G., Cho, I. H., Chung, D. H. et al., STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. *FASEB J.* 2005, *19*, 1296–1298.

[77] Boreddy, S. R., Sahu, R. P., Srivastava, S. K., Benzyl isothiocyanate suppresses pancreatic tumor angiogenesis and invasion by inhibiting HIF-alpha/VEGF/Rho-GTPases: pivotal role of STAT-3. *PLoS One* 2011, *6*, e25799.

[78] Warin, R., Xiao, D., Arlotti, J. A., Bommareddy, A. et al., Inhibition of human breast cancer xenograft growth by cruciferous vegetable constituent benzyl isothiocyanate. *Mol. Carcinog.* 2010, *49*, 500–507.

[79] Wu, C. L., Huang, A. C., Yang, J. S., Liao, C. L. et al., Benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC)-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of caspase-3, mitochondria dysfunction and nitric oxide (NO) in human osteogenic sarcoma U-2 OS cells. *J. Orthop. Res.* 2011, *29*, 1199–1209.

[80] Wu, X., Zhu, Y., Yan, H., Liu, B. et al., Isothiocyanates induce oxidative stress and suppress the metastasis potential of human non-small cell lung cancer cells. *BMC Cancer* 2010, *10*, 269.

[81] Miyoshi, N., Takabayashi, S., Osawa, T., Nakamura, Y., Benzyl isothiocyanate inhibits excessive superoxide generation in inflammatory leukocytes: implication for prevention against inflammation-related carcinogenesis. *Carcinogenesis* 2004, *25*, 567–575.

[82] Zhu, Y., Zhuang, J. X., Wang, Q., Zhang, H. Y. et al., Inhibitory effect of benzyl isothiocyanate on proliferation in vitro of human glioma cells. *Asian Pac. J. Cancer Prev.* 2013, *14*, 2607–2610.

[83] Kawakami, M., Harada, N., Hiratsuka, M., Kawai, K. et al., Dietary isothiocyanates modify mitochondrial functions through their electrophilic reaction. *Biosci. Biotechnol. Biochem.* 2005, *69*, 2439–2444.

[84] Zhou, T., Li, G., Cao, B., Liu, L. et al., Downregulation of Mcl-1 through inhibition of translation contributes to benzyl isothiocyanate-induced cell cycle arrest and apoptosis in human leukemia cells. *Cell Death Dis.* 2013, *4*, e515.

[85] Kim, E. J., Hong, J. E., Eom, S. J., Lee, J. Y. et al., Oral administration of benzyl-isothiocyanate inhibits solid tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. *Breast Cancer Res. Treat.* 2011, *130*, 61–71.

[86] Sehrawat, A., Kim, S. H., Vogt, A., Singh, S. V., Suppression of FOXQ1 in benzyl isothiocyanate-mediated inhibition of epithelial-mesenchymal transition in human breast cancer cells. *Carcinogenesis* 2013, *34*, 864–873.

[87] Kim, S. H., Sehrawat, A., Singh, S. V., Dietary chemopreventative benzyl isothiocyanate inhibits breast cancer stem cells in vitro and in vivo. *Cancer Prev. Res.* 2013, *6*, 782–790.

[88] Kim, S. H., Sehrawat, A., Singh, S. V., Notch2 activation by benzyl isothiocyanate impedes its inhibitory effect on breast cancer cell migration. *Breast Cancer Res. Treat.* 2012, *134*, 1067–1079.

[89] Qhattal, H. S., Wang, S., Salihima, T., Srivastava, S. K. et al., Nanoemulsions of cancer chemopreventive agent benzyl isothiocyanate display enhanced solubility, dissolution, and permeability. *J. Agric. Food Chem.* 2011, *59*, 12396–12404.

[90] Lewerenz, H. J., Bleyl, D. W., Plass, R., Subacute oral toxicity study of benzyl isothiocyanate in rats. *Die Nahrung* 1992, *36*, 190–198.

[91] Hecht, S. S., Chung, F. L., Richie, J. P. Jr., Akerkar, S. A. et al., Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. *Cancer Epidemiol. Biomarkers Prev.* 1995, *4*, 877–884.

[92] Chung, F. L., Morse, M. A., Eklind, K. I., Lewis, J., Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. *Cancer Epidemiol. Biomarkers Prev.* 1992, *1*, 383–388.

[93] Guo, Z., Smith, T. J., Wang, E., Eklind, K. I. et al., Structure-activity relationships of arylalkyl isothiocyanates for the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolism and the modulation of xenobiotic-metabolizing enzymes in rats and mice. *Carcinogenesis* 1993, *14*, 1167–1173.

[94] Gupta, P., Adkins, C., Lockman, P., Srivastava, S., Metastasis of breast tumor cells to brain is suppressed by phenethyl isothiocyanate in a novel in vivo metastasis model. *PLoS One* 2013, *8*, e67278.

[95] Gupta, P., Srivastava, S. K., Antitumor activity of phenethyl isothiocyanate in HER2-positive breast cancer models. *BMC Med.* 2012, *10*, 80.

[96] Trachootham, D., Zhou, Y., Zhang, H., Demizu, Y. et al., Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell* 2006, *10*, 241–252.

[97] Xiao, D., Singh, S. V., Phenethyl isothiocyanate sensitizes androgen-independent human prostate cancer cells to docetaxel-induced apoptosis in vitro and in vivo. *Pharm. Res.* 2010, *27*, 722–731.

[98] Chen, G., Chen, Z., Hu, Y., Huang, P., Inhibition of mitochondrial respiration and rapid depletion of mitochondrial glutathione by beta-phenethyl isothiocyanate: mechanisms for anti-leukemia activity. *Antioxid. Redox Signal.* 2011, *15*, 2911–2921.

[99] Syed Alwi, S. S., Cavell, B. E., Donlevy, A., Packham, G., Differential induction of apoptosis in human breast cancer cell

lines by phenethyl isothiocyanate, a glutathione depleting agent. *Cell Stress Chaperones* 2012, *17*, 529–538.

[100] Powolny, A. A., Singh, S. V., Differential response of normal (PrEC) and cancerous human prostate cells (PC-3) to phenethyl isothiocyanate-mediated changes in expression of antioxidant defense genes. *Pharm. Res.* 2010, *27*, 2766–2775.

[101] Chen, Y. R., Wang, W., Kong, A. N., Tan, T. H., Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. *J. Biol. Chem.* 1998, *273*, 1769–1775.

[102] Yang, Y. M., Conaway, C. C., Chiao, J. W., Wang, C. X. et al., Inhibition of benzo(a)pyrene-induced lung tumorigenesis in A/J mice by dietary N-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res.* 2002, *62*, 2–7.

[103] Lai, K. C., Hsu, S. C., Kuo, C. L., Ip, S. W. et al., Phenethyl isothiocyanate inhibited tumor migration and invasion via suppressing multiple signal transduction pathways in human colon cancer HT29 cells. *J. Agric. Food Chem.* 2010, 11148–11155.

[104] Hwang, E. S., Lee, H. J., Phenylethyl isothiocyanate and its *N*-acetylcysteine conjugate suppress the metastasis of SK-Hep1 human hepatoma cells. *J. Nutr. Biochem.* 2006, *17*, 837–846.

[105] Xiao, D., Singh, S. V., Phenethyl isothiocyanate inhibits angiogenesis in vitro and ex vivo. *Cancer Res.* 2007, *67*, 2239–2246.

[106] Wang, X. H., Cavell, B. E., Syed Alwi, S. S., Packham, G., Inhibition of hypoxia inducible factor by phenethyl isothiocyanate. *Biochem. Pharmacol.* 2009, *78*, 261–272.

[107] Mi, L., Hood, B. L., Stewart, N. A., Xiao, Z. et al., Identification of potential protein targets of isothiocyanates by proteomics. *Chem. Res. Toxicol.* 2011, *24*, 1735–1743.

[108] Suzuki, S., Dobashi, Y., Minato, H., Tajiri, R. et al., EGFR and HER2-Akt-mTOR signaling pathways are activated in subgroups of salivary gland carcinomas. *Virchows Arch.* 2012, *461*, 271–282.

[109] Loganathan, S., Kandala, P. K., Gupta, P., Srivastava, S. K., Inhibition of EGFR-AKT axis results in the suppression of ovarian tumors in vitro and in preclinical mouse model. *PLoS One* 2012, *7*, e43577.

[110] Kang, L., Wang, Z. Y., Breast cancer cell growth inhibition by phenethyl isothiocyanate is associated with down-regulation of oestrogen receptor-alpha36. *J. Cell. Mol. Med.* 2010, *14*, 1485–1493.

[111] Syed Alwi, S. S., Cavell, B. E., Telang, U., Morris, M. E. et al., In vivo modulation of 4E binding protein 1 (4E-BP1) phosphorylation by watercress: a pilot study. *Br. J. Nutr.* 2010, *104*, 1288–1296.

[112] Izzotti, A., Larghero, P., Balansky, R., Pfeffer, U. et al., Interplay between histopathological alterations, cigarette smoke and chemopreventive agents in defining microRNA profiles in mouse lung. *Mutat. Res.* 2011, *717*, 17–24.

[113] Huong, L. D., Shin, J. A., Choi, E. S., Cho, N. P. et al., Beta-phenethyl isothiocyanate induces death receptor 5 to induce apoptosis in human oral cancer cells via p38. *Oral Dis.* 2012, *18*, 513–519.

[114] Huong le, D., Shim, J. H., Choi, K. H., Shin, J. A. et al., Effect of beta-phenylethyl isothiocyanate from cruciferous vegetables on growth inhibition and apoptosis of cervical cancer cells through the induction of death receptors 4 and 5. *J. Agric. Food Chem.* 2011, *59*, 8124–8131.

[115] Pullar, J. M., Thomson, S. J., King, M. J., Turnbull, C. I. et al., The chemopreventive agent phenethyl isothiocyanate sensitizes cells to Fas-mediated apoptosis. *Carcinogenesis* 2004, *25*, 765–772.

[116] Chen, P. Y., Lin, K. C., Lin, J. P., Tang, N. Y. et al., Phenethyl isothiocyanate (PEITC) inhibits the growth of human oral squamous carcinoma HSC-3 cells through G(0)/G(1) phase arrest and mitochondria-mediated apoptotic cell death. *Evid. Based Complement. Altern. Med.* 2012, *2012*, 718320.

[117] Tang, N. Y., Huang, Y. T., Yu, C. S., Ko, Y. C. et al., Phenethyl isothiocyanate (PEITC) promotes G2/M phase arrest via p53 expression and induces apoptosis through caspase- and mitochondria-dependent signaling pathways in human prostate cancer DU 145 cells. *Anticancer Res.* 2011, *31*, 1691–1702.

[118] Lee, J. W., Cho, M. K., Phenethyl isothiocyanate induced apoptosis via down regulation of Bcl-2/XIAP and triggering of the mitochondrial pathway in MCF-7 cells. *Arch. Pharm. Res.* 2008, *31*, 1604–1612.

[119] Xiao, D., Lew, K. L., Zeng, Y., Xiao, H. et al., Phenethyl isothiocyanate-induced apoptosis in PC-3 human prostate cancer cells is mediated by reactive oxygen species-dependent disruption of the mitochondrial membrane potential. *Carcinogenesis* 2006, *27*, 2223–2234.

[120] Wang, X., Di Pasqua, A. J., Govind, S., McCracken, E. et al., Selective depletion of mutant p53 by cancer chemopreventive isothiocyanates and their structure-activity relationships. *J. Med. Chem.* 2011, *54*, 809–816.

[121] Chiao, J. W., Wu, H., Ramaswamy, G., Conaway, C. C. et al., Ingestion of an isothiocyanate metabolite from cruciferous vegetables inhibits growth of human prostate cancer cell xenografts by apoptosis and cell cycle arrest. *Carcinogenesis* 2004, *25*, 1403–1408.

[122] Saw, C. L., Cintron, M., Wu, T. Y., Guo, Y. et al., Pharmacodynamics of dietary phytochemical indoles I3C and DIM: induction of Nrf2-mediated phase II drug metabolizing and antioxidant genes and synergism with isothiocyanates. *Biopharm. Drug Dispos.* 2011, *32*, 289–300.

[123] Moon, Y. J., Brazeau, D. A., Morris, M. E., Dietary phenethyl isothiocyanate alters gene expression in human breast cancer cells. *Evid. Based Complement. Altern. Med.* 2011, *2011*, 462525.

[124] Pawlik, A., Szczepanski, M. A., Klimaszewska, A., Gackowska, L. et al., Phenethyl isothiocyanate-induced cytoskeletal changes and cell death in lung cancer cells. *Food Chem. Toxicol.* 2012, *50*, 3577–3594.

[125] Sakao, K., Desineni, S., Hahm, E. R., Singh, S. V., Phenethyl isothiocyanate suppresses inhibitor of apoptosis family

protein expression in prostate cancer cells in culture and in vivo. *Prostate* 2012, *72*, 1104–1116.

[126] Ji, Y., Kuo, Y., Morris, M. E., Pharmacokinetics of dietary phenethyl isothiocyanate in rats. *Pharm. Res.* 2005, *22*, 1658–1666.

[127] Conaway, C. C., Jiao, D., Kohri, T., Liebes, L. et al., Disposition and pharmacokinetics of phenethyl isothiocyanate and 6-phenylhexyl isothiocyanate in F344 rats. *Drug Metab. Dispos.* 1999, *27*, 13–20.

[128] Gao, N., Budhraja, A., Cheng, S., Liu, E. H. et al., Phenethyl isothiocyanate exhibits antileukemic activity in vitro and in vivo by inactivation of Akt and activation of JNK pathways. *Cell Death Dis.* 2011, *2*, e140.

[129] Tsou, M. F., Tien, N., Lu, C. C., Chiang, J. H. et al., Phenethyl isothiocyanate promotes immune responses in normal BALB/c mice, inhibits murine leukemia WEHI-3 cells, and stimulates immunomodulations in vivo. *Environ. Toxicol.* 2013, *28*, 127–136.

[130] Li, Y., Wang, E. J., Chen, L., Stein, A. P. et al., Effects of phenethyl isothiocyanate on acetaminophen metabolism and hepatotoxicity in mice. *Toxicol. Appl. Pharmacol.* 1997, *144*, 306–314.

[131] Mi, L., Xiao, Z., Hood, B. L., Dakshanamurthy, S. et al., Covalent binding to tubulin by isothiocyanates. A mechanism of cell growth arrest and apoptosis. *J. Biol. Chem.* 2008, *283*, 22136–22146.

[132] Myzak, M. C., Hardin, K., Wang, R., Dashwood, R. H. et al., Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. *Carcinogenesis* 2006, *27*, 811–819.

[133] Dashwood, R. H., Ho, E., Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin. Cancer Biol.* 2007, *17*, 363–369.

[134] Wang, F., Shan, Y., Sulforaphane retards the growth of UM-UC-3 xenographs, induces apoptosis, and reduces survivin in athymic mice. *Nutr. Res.* 2012, *32*, 374–380.

[135] Xu, C., Shen, G., Chen, C., Gelinas, C. et al., Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. *Oncogene* 2005, *24*, 4486–4495.

[136] Pledgie-Tracy, A., Sobolewski, M. D., Davidson, N. E., Sulforaphane induces cell type-specific apoptosis in human breast cancer cell lines. *Mol. Cancer Ther.* 2007, *6*, 1013–1021.

[137] Rajendran, P., Kidane, A. I., Yu, T. W., Dashwood, W. M. et al., HDAC turnover, CtIP acetylation and dysregulated DNA damage signaling in colon cancer cells treated with sulforaphane and related dietary isothiocyanates. *Epigenetics* 2013, *8*, 612–623.

[138] Hsu, A., Wong, C. P., Yu, Z., Williams, D. E. et al., Promoter de-methylation of cyclin D2 by sulforaphane in prostate cancer cells. *Clin. Epigenetics* 2011, *3*, 3.

[139] Jackson, S. J., Singletary, K. W., Venema, R. C., Sulforaphane suppresses angiogenesis and disrupts endothelial mitotic progression and microtubule polymerization. *Vasc. Pharmacol.* 2007, *46*, 77–84.

[140] Bertl, E., Bartsch, H., Gerhauser, C., Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. *Mol. Cancer Ther.* 2006, *5*, 575–585.

[141] Davis, R., Singh, K. P., Kurzrock, R., Shankar, S., Sulforaphane inhibits angiogenesis through activation of FOXO transcription factors. *Oncol. Rep.* 2009, *22*, 1473–1478.

[142] Choi, S., Lew, K. L., Xiao, H., Herman-Antosiewicz, A. et al., D,L-Sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. *Carcinogenesis* 2007, *28*, 151–162.

[143] Li, S. H., Fu, J., Watkins, D. N., Srivastava, R. K. et al., Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog-GLI pathway. *Mol. Cell. Biochem.* 2013, *373*, 217–227.

[144] Singh, S. V., Srivastava, S. K., Choi, S., Lew, K. L. et al., Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. *J. Biol. Chem.* 2005, *280*, 19911–19924.

[145] Hsu, Y. C., Chang, S. J., Wang, M. Y., Chen, Y. L. et al., Growth inhibition and apoptosis of neuroblastoma cells through ROS-independent MEK/ERK activation by sulforaphane. *Cell Biochem. Biophys.* 2013, *66*, 765–774.

[146] Shan, Y., Sun, C., Zhao, X., Wu, K. et al., Effect of sulforaphane on cell growth, G(0)/G(1) phase cell progression and apoptosis in human bladder cancer T24 cells. *Int. J. Oncol.* 2006, *29*, 883–888.

[147] Gamet-Payrastre, L., Li, P., Lumeau, S., Cassar, G. et al., Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res.* 2000, *60*, 1426–1433.

[148] Suppipat, K., Park, C. S., Shen, Y., Zhu, X. et al., Sulforaphane induces cell cycle arrest and apoptosis in acute lymphoblastic leukemia cells. *PLoS One* 2012, *7*, e51251.

[149] Shen, G., Xu, C., Chen, C., Hebbar, V. et al., p53-independent G1 cell cycle arrest of human colon carcinoma cells HT-29 by sulforaphane is associated with induction of p21CIP1 and inhibition of expression of cyclin D1. *Cancer Chemother. Pharmacol.* 2006, *57*, 317–327.

[150] Jakubikova, J., Sedlak, J., Mithen, R., Bao, Y., Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G2/M arrest and cell death in Caco-2 cells. *Biochem. Pharmacol.* 2005, *69*, 1543–1552.

[151] Jee, H. G., Lee, K. E., Kim, J. B., Shin, H. K. et al., Sulforaphane inhibits oral carcinoma cell migration and invasion in vitro. *Phytother. Res.* 2011, *25*, 1623–1628.

[152] Kanematsu, S., Yoshizawa, K., Uehara, N., Miki, H. et al., Sulforaphane inhibits the growth of KPL-1 human breast cancer cells in vitro and suppresses the growth and metastasis of orthotopically transplanted KPL-1 cells in female athymic mice. *Oncol. Rep.* 2011, *26*, 603–608.

[153] Shan, Y., Zhang, L., Bao, Y., Li, B. et al., Epithelial-mesenchymal transition, a novel target of sulforaphane via COX-2/MMP2, 9/Snail, ZEB1 and miR-200c/ZEB1 pathways

in human bladder cancer cells. *J. Nutr. Biochem.* 2013, *24*, 1062–1069.

[154] Gerhauser, C., Epigenetic impact of dietary isothiocyanates in cancer chemoprevention. *Curr. Opin. Clin. Nutr. Metab. Care* 2013, *16*, 405–410.

[155] Hu, R., Hebbar, V., Kim, B. R., Chen, C. et al., In vivo pharmacokinetics and regulation of gene expression profiles by isothiocyanate sulforaphane in the rat. *J. Pharmacol. Exp. Ther.* 2004, *310*, 263–271.

[156] Cornblatt, B. S., Ye, L., Dinkova-Kostova, A. T., Erb, M. et al., Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis* 2007, *28*, 1485–1490.

[157] Hu, R., Khor, T. O., Shen, G., Jeong, W. S. et al., Cancer chemoprevention of intestinal polyposis in ApcMin/ +mice by sulforaphane, a natural product derived from cruciferous vegetable. *Carcinogenesis* 2006, *27*, 2038–2046.

[158] Priya, D. K. D., Gayathri, R., Gunassekaran, G. R., Murugan, S. et al., Inhibitory effect of sulforaphane against benzo(a)pyrene induced lung cancer by modulation of biochemical signatures in female Swiss albino mice. *Asian J. Biochem.* 2011, *6*, 395–405.

[159] Zhao, H. D., Zhang, F., Shen, G., Li, Y. B. et al., Sulforaphane protects liver injury induced by intestinal ischemia reperfusion through Nrf2-ARE pathway. *World J. Gastroenterol.* 2010, *16*, 3002–3010.

[160] Jiao, D., Ho, C. T., Foiles, P., Chung, F. L., Identification and quantification of the N-acetylcysteine conjugate of allyl isothiocyanate in human urine after ingestion of mustard. *Cancer Epidemiol. Biomarkers Prev.* 1994, *3*, 487–492.

[161] Charron, C. S., Clevidence, B. A., Albaugh, G. A., Kramer, M. H. et al., Assessment of DNA damage and repair in adults consuming allyl isothiocyanate or Brassica vegetables. *J. Nutr. Biochem.* 2013, *24*, 894–902.

[162] Smith, T., Musk, S. R., Johnson, I. T., Allyl isothiocyanate selectively kills undifferentiated HT29 cells in vitro and suppresses aberrant crypt foci in the colonic mucosa of rats. *Biochem. Soc. Trans.* 1996, *24*, 381S.

[163] Smith, T. K., Lund, E. K., Parker, M. L., Clarke, R. G. et al., Allyl-isothiocyanate causes mitotic block, loss of cell adhesion and disrupted cytoskeletal structure in HT29 cells. *Carcinogenesis* 2004, *25*, 1409–1415.

[164] Xu, C., Shen, G., Yuan, X., Kim, J. H. et al., ERK and JNK signaling pathways are involved in the regulation of activator protein 1 and cell death elicited by three isothiocyanates in human prostate cancer PC-3 cells. *Carcinogenesis* 2006, *27*, 437–445.

[165] Tsai, S. C., Huang, W. W., Huang, W. C., Lu, C. C. et al., ERK-modulated intrinsic signaling and G(2)/M phase arrest contribute to the induction of apoptotic death by allyl isothiocyanate in MDA-MB-468 human breast adenocarcinoma cells. *Int. J. Oncol.* 2012, *41*, 2065–2072.

[166] Geng, F., Tang, L., Li, Y., Yang, L. et al., Allyl isothiocyanate arrests cancer cells in mitosis, and mitotic arrest in turn leads to apoptosis via Bcl-2 protein phosphorylation. *J. Biol. Chem.* 2011, *286*, 32259–32267.

[167] Srivastava, S. K., Xiao, D., Lew, K. L., Hershberger, P. et al., Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits growth of PC-3 human prostate cancer xenografts in vivo. *Carcinogenesis* 2003, *24*, 1665–1670.

[168] Hwang, E. S., Lee, H. J., Allyl isothiocyanate and its N-acetylcysteine conjugate suppress metastasis via inhibition of invasion, migration, and matrix metalloproteinase-2/-9 activities in SK-Hep 1 human hepatoma cells. *Exp. Biol. Med.* 2006, *231*, 421–430.

[169] Kumar, A., D'Souza, S. S., Tickoo, S., Salimath, B. P. et al., Antiangiogenic and proapoptotic activities of allyl isothiocyanate inhibit ascites tumor growth in vivo. *Integr. Cancer Ther.* 2009, *8*, 75–87.

[170] Thejass, P., Kuttan, G., Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) inhibit tumour-specific angiogenesis by downregulating nitric oxide (NO) and tumour necrosis factor-alpha (TNF-alpha) production. *Nitric Oxide* 2007, *16*, 247–257.

[171] Imaizumi, K., Sato, S., Sakakibara, Y., Mori, S. et al., Allyl isothiocyanate-induced changes in the distribution of white blood cells in rats. *J. Toxicol. Sci.* 2010, *35*, 583–589.

[172] Manesh, C., Kuttan, G., Effect of naturally occurring isothiocyanates on the immune system. *Immunopharmacol. Immunotoxicol.* 2003, *25*, 451–459.

[173] Lewerenz, H. J., Plass, R., Macholz, R., Effect of allyl isothiocyanate on hepatic monooxygenases and serum transferases in rats. *Toxicol. Lett.* 1988, *44*, 65–70.

[174] Masutomi, N., Toyoda, K., Shibutani, M., Niho, N. et al., Toxic effects of benzyl and allyl isothiocyanates and benzyl-isoform specific metabolites in the urinary bladder after a single intravesical application to rats. *Toxicol. Pathol.* 2001, *29*, 617–622.

[175] Sawai, Y., Murata, H., Horii, M., Koto, K. et al., Effectiveness of sulforaphane as a radiosensitizer for murine osteosarcoma cells. *Oncol. Rep.* 2013, *29*, 941–945.

[176] Wu, W. J., Zhang, Y., Zeng, Z. L., Li, X. B. et al., beta-phenylethyl isothiocyanate reverses platinum resistance by a GSH-dependent mechanism in cancer cells with epithelial-mesenchymal transition phenotype. *Biochem. Pharmacol.* 2013, *85*, 486–496.

[177] Chan, D. K., Miskimins, W. K., Metformin and phenethyl isothiocyanate combined treatment in vitro is cytotoxic to ovarian cancer cultures. *J. Ovarian Res.* 2012, *5*, 19.

[178] Hu, Y., Lu, W., Chen, G., Zhang, H. et al., Overcoming resistance to histone deacetylase inhibitors in human leukemia with the redox modulating compound beta-phenylethyl isothiocyanate. *Blood* 2010, *116*, 2732–2741.

[179] Rausch, V., Liu, L., Kallifatidis, G., Baumann, B. et al., Synergistic activity of sorafenib and sulforaphane abolishes pancreatic cancer stem cell characteristics. *Cancer Res.* 2010, *70*, 5004–5013.

[180] Wang, X., Govind, S., Sajankila, S. P., Mi, L. et al., Phenethyl isothiocyanate sensitizes human cervical cancer cells to apoptosis induced by cisplatin. *Mol. Nutr. Food Res.* 2011, *55*, 1572–1581.

[181] Wang, X. F., Wu, D. M., Li, B. X., Lu, Y. J. et al., Synergistic inhibitory effect of sulforaphane and 5-fluorouracil in high and low metastasis cell lines of salivary gland adenoid cystic carcinoma. *Phytother. Res.* 2009, *23*, 303–307.

[182] el-Bayoumy, K., Upadhyaya, P., Desai, D. H., Amin, S. et al., Effects of 1,4-phenylenebis(methylene)selenocyanate, phenethyl isothiocyanate, indole-3-carbinol, and d-limonene individually and in combination on the tumorigenicity of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Anticancer Res.* 1996, *16*, 2709–2712.

[183] Kotowski, U., Heiduschka, G., Brunner, M., Czembirek, C. et al., Radiosensitization of head and neck cancer cells by the phytochemical agent sulforaphane. *Strahlentherapie und Onkologie* 2011, *187*, 575–580.

[184] Doudican, N. A., Wen, S. Y., Mazumder, A., Orlow, S. J., Sulforaphane synergistically enhances the cytotoxicity of arsenic trioxide in multiple myeloma cells via stress-mediated pathways. *Oncol. Rep.* 2012, *28*, 1851–1858.

[185] Jiang, H., Shang, X., Wu, H., Huang, G. et al., Combination treatment with resveratrol and sulforaphane induces apoptosis in human U251 glioma cells. *Neurochem. Res.* 2010, *35*, 152–161.

[186] Srivastava, R. K., Tang, S. N., Zhu, W., Meeker, D. et al., Sulforaphane synergizes with quercetin to inhibit self-renewal capacity of pancreatic cancer stem cells. *Front. Biosci.* 2011, *3*, 515–528.

[187] Pradhan, S. J., Mishra, R., Sharma, P., Kundu, G. C., Quercetin and sulforaphane in combination suppress the progression of melanoma through the down-regulation of matrix metalloproteinase-9. *Exp. Ther. Med.* 2010, *1*, 915–920.

[188] Sheridan, J. P., Marsters, S. A., Pitti, R. M., Gurney, A. et al., Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* 1997, *277*, 818–821.

[189] Koschny, R., Walczak, H., Ganten, T. M., The promise of TRAIL—potential and risks of a novel anticancer therapy. *J. Mol. Med.* 2007, *85*, 923–935.

[190] Shankar, S., Srivastava, R. K., Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications. *Drug Resist. Updat.* 2004, *7*, 139–156.

[191] Wicker, C. A., Sahu, R. P., Kulkarni-Datar, K., Srivastava, S. K. et al., BITC sensitizes pancreatic adenocarcinomas to TRAIL-induced apoptosis. *Cancer Growth Metastasis* 2010, *2009*, 45–55.

[192] Di Pasqua, A. J., Hong, C., Wu, M. Y., McCracken, E. et al., Sensitization of non-small cell lung cancer cells to cisplatin by naturally occurring isothiocyanates. *Chem. Res. Toxicol.* 2010, *23*, 1307–1309.

[193] Fimognari, C., Lenzi, M., Sciuscio, D., Cantelli-Forti, G. et al., Combination of doxorubicin and sulforaphane for reversing doxorubicin-resistant phenotype in mouse fibroblasts with p53Ser220 mutation. *Ann. N. Y. Acad. Sci.* 2007, *1095*, 62–69.

[194] Mukherjee, S., Dey, S., Bhattacharya, R. K., Roy, M., Isothiocyanates sensitize the effect of chemotherapeutic drugs via modulation of protein kinase C and telomerase in cervical cancer cells. *Mol. Cell. Biochem.* 2009, *330*, 9–22.

[195] Liu, K., Cang, S., Ma, Y., Chiao, J. W., Synergistic effect of paclitaxel and epigenetic agent phenethyl isothiocyanate on growth inhibition, cell cycle arrest and apoptosis in breast cancer cells. *Cancer Cell Int.* 2013, *13*, 10.

[196] Kaminski, B. M., Weigert, A., Brune, B., Schumacher, M. et al., Sulforaphane potentiates oxaliplatin-induced cell growth inhibition in colorectal cancer cells via induction of different modes of cell death. *Cancer Chemother. Pharmacol.* 2011, *67*, 1167–1178.

[197] Kallifatidis, G., Rausch, V., Baumann, B., Apel, A. et al., Sulforaphane targets pancreatic tumour-initiating cells by NF-kappaB-induced antiapoptotic signalling. *Gut* 2009, *58*, 949–963.

[198] Milczarek, M., Misiewicz-Krzeminska, I., Lubelska, K., Wiktorska, K., Combination treatment with 5-fluorouracil and isothiocyanates shows an antagonistic effect in Chinese hamster fibroblast cells line-V79. *Acta Pol. Pharm.* 2011, *68*, 331–342.

[199] Kandala, P. K., Srivastava, S. K., DIMming ovarian cancer growth. *Curr. Drug Targets* 2012, *13*, 1869–1875.

[200] Pappa, G., Strathmann, J., Lowinger, M., Bartsch, H. et al., Quantitative combination effects between sulforaphane and 3,3′-diindolylmethane on proliferation of human colon cancer cells in vitro. *Carcinogenesis* 2007, *28*, 1471–1477.

[201] Hutzen, B., Willis, W., Jones, S., Cen, L. et al., Dietary agent, benzyl isothiocyanate inhibits signal transducer and activator of transcription 3 phosphorylation and collaborates with sulforaphane in the growth suppression of PANC-1 cancer cells. *Cancer Cell Int.* 2009, *9*, 24.

[202] Hecht, S. S., Kenney, P. M., Wang, M., Trushin, N. et al., Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/J mice by benzo[a]pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett.* 2000, *150*, 49–56.

[203] Shishodia, S., Chaturvedi, M. M., Aggarwal, B. B., Role of curcumin in cancer therapy. *Curr. Probl. Cancer* 2007, *31*, 243–305.

[204] Sahu, R. P., Batra, S., Srivastava, S. K., Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. *Br. J. Cancer* 2009, *100*, 1425–1433.

[205] Cheung, K. L., Khor, T. O., Kong, A. N., Synergistic effect of combination of phenethyl isothiocyanate and sulforaphane or curcumin and sulforaphane in the inhibition of inflammation. *Pharm. Res.* 2009, *26*, 224–231.

[206] Thakkar, A., Sutaria, D., Grandhi, B. K., Wang, J. et al., The molecular mechanism of action of aspirin, curcumin and sulforaphane combinations in the chemoprevention of pancreatic cancer. *Oncol. Rep.* 2013, *29*, 1671–1677.

[207] Barve, A., Khor, T. O., Hao, X., Keum, Y. S. et al., Murine prostate cancer inhibition by dietary phytochemicals—curcumin and phenyethylisothiocyanate. *Pharm. Res.* 2008, *25*, 2181–2189.

[208] Khor, T. O., Keum, Y. S., Lin, W., Kim, J. H. et al., Combined inhibitory effects of curcumin and phenethyl isothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice. *Cancer Res.* 2006, *66*, 613–621.

[209] Kim, J. H., Xu, C., Keum, Y. S., Reddy, B. et al., Inhibition of EGFR signaling in human prostate cancer PC-3 cells by combination treatment with beta-phenylethyl isothiocyanate and curcumin. *Carcinogenesis* 2006, *27*, 475–482.

[210] Singh, B. N., Shankar, S., Srivastava, R. K., Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. *Biochem. Pharmacol.* 2011, *82*, 1807–1821.

[211] Nair, S., Barve, A., Khor, T. O., Shen, G. X. et al., Regulation of Nrf2- and AP-1-mediated gene expression by epigallocatechin-3-gallate and sulforaphane in prostate of Nrf2-knockout or C57BL/6J mice and PC-3 AP-1 human prostate cancer cells. *Acta Pharmacol. Sin.* 2010, *31*, 1223–1240.

[212] Chen, H., Landen, C. N., Li, Y., Alvarez, R. D. et al., Epigallocatechin gallate and sulforaphane combination treatment induce apoptosis in paclitaxel-resistant ovarian cancer cells through hTERT and Bcl-2 down-regulation. *Exp. Cell Res.* 2013, *319*, 697–706.

[213] De Nicola, G. R., Montaut, S., Rollin, P., Nyegue, M., Stability of benzylic-type isothiocyanates in hydrodistillation-mimicking conditions. *J. Agric. Food Chem.* 2013, *61*, 137–142.

[214] Franklin, S. J., Dickinson, S. E., Karlage, K. L., Bowden, G. T. et al., Stability of sulforaphane for topical formulation. *Drug Dev. Ind. Pharm.* 2013.

