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REVIEW

Sulforaphane as an anticancer molecule: mechanisms of action, synergistic effects, enhancement of drug safety, and delivery systems

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Abstract Sulforaphane is an isothiocyanate compound that has been derived from cruciferous vegetables. It was shown in numerous studies to be active against multiple cancer types including pancreatic, prostate, breast, lung, cervical, and colorectal cancers. Sulforaphane exerts its therapeutics action by a variety of mechanisms, such as by detoxifying carcinogens and oxidants through blockage of phase I metabolic enzymes, and by arresting cell cycle in the G2/M and G1 phase to inhibit cell proliferation. The most striking observation was the ability of sulforaphane to potentiate the activity of several classes of anticancer agents including paclitaxel, docetaxel, and gemcitabine through additive and synergistic effects. Although a good number of reviews have reported on the mechanisms by which sulforaphane exerts its anticancer activity, a comprehensive review on the synergistic effect of sulforaphane and its delivery strategies is lacking. Therefore, the aim of the current review was to provide a summary of the studies that have been reported on the activity enhancement effect of sulforaphane in combination with other anticancer therapies. Also provided is a summary of the strategies that have been developed for the delivery of sulforaphane.

Keywords Cancer · Drug delivery systems · Sulforaphane · Combination therapy · Synergism · Isothiocyanates

Introduction

Sulforaphane (4-methylsulfinybutyl isothiocyanate) is an oily sulfur-containing isothiocyanate phytochemical that was derived from cruciferous vegetables, such as brussels and broccoli sprouts, cabbage, and cauliflower (Fig. 1). Broccoli sprout has the highest sulforaphane content at 1153 mg of sulforaphane per 100 g, whereas mature broccoli contains 44–171 mg of sulforaphane /100 g dry weight (Nakagawa et al. 2006). Sulforaphane can be isolated by solvent extraction process or by macroporous resins with a high ratio of adsorption and desorption. Sulforaphane, as with the other isothiocyanates (ITC), is stored in plants as glucosinolates. During extraction, the glucosinolates are converted into sulforaphane via the myrosinase catalyzed process (Fig. 1). In normal plants, myrosinase enzyme coexists with glucosinolates but stays physically separated (Zhang and Tang 2007).

Even though sulforaphane was isolated and identified in 1959, it received considerable attention only in 1992 when Prochaska and her colleagues (Prochaska et al. 1992) developed a method for screening extracts of fruits and vegetables that can induce phase 2 enzymes. Sulforaphane was found to be a very potent phase 2 enzymes Inducer, which detoxify electrophiles to protect animal from carcinogenesis (Zhang et al. 1992). Later, sulforaphane was shown to be working

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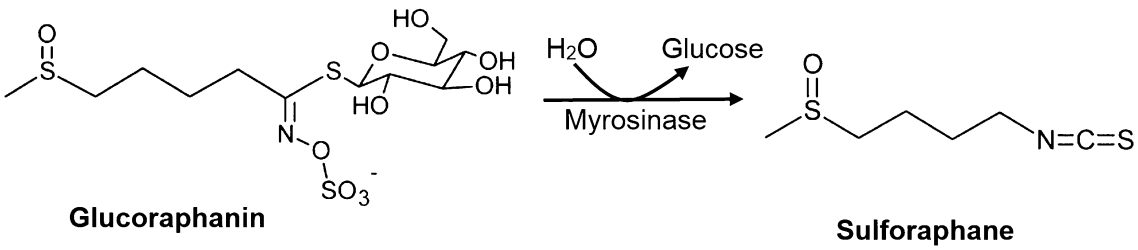

Fig. 1 Conversion of glucoraphanin to sulforaphane

as an anti-inflammatory and antioxidative agent (Fahey and Talalay 1999; Cheung and Kong 2010).

Structurally, sulforaphane contains a unique configuration, which is crucial for its phase 2 enzymes induction activity. In order to fabricate a more potent phase 2 enzymes inducer, over 40 sulforaphane analogs were synthesized by converting sulfoxide to sulfone or sulfide in methylthiol group, replacing sulfoxide with the methylene group or carbonyl group, changing the number of methylene units to 3 or 5 from 4, modifying the methylene bridge rigidity and by conversion of –N=C=S group to various dithiocarbamate structures. In all cases, there was no improvement in phase 2 enzymes induction activity; rather activity was reduced in some cases proving the importance of sulforaphane structure (Posner et al. 1994; Moriarty et al. 2006; Zhang and Tang 2007). In most cases, unmodified sulforaphane was therefore investigated for its therapeutic activity without modification. Sulforaphane exerts its therapeutic effect by activating multiple mechanisms including Nrf2-mediated induction of phase 2 detoxification enzymes, cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis (Juge et al. 2007). Although a good number of reviews have reported on the mechanisms by which sulforaphane exerts its anticancer activity (Fimognari and Hrelia 2007; Juge et al. 2007; Clarke et al. 2008; Sestili and Fimognari 2015), a comprehensive review on the synergistic effect of sulforaphane and its delivery strategies is lacking. Therefore, the aim of the current review was to provide a summary of the studies that have reported on the activity enhancement effect of sulforaphane in combination with other anticancer therapies. Also provided is a summary of the strategies that have been developed to date for the entrapment and delivery of sulforaphane.

Mechanism of anticancer activity

A plethora of studied have been reported on the anticancer activity of sulforaphane against a broad range of cancers including prostate, pancreatic, breast, lung, cervical, bladder, colorectal, melanoma, and ovarian cancers (Cornblatt et al. 2007; Kallifatidis et al. 2009; Sharma et al. 2011; Wiczk et al. 1823; Li et al. 2013; Jo et al. 2014; Atwell et al. 2015a, b; Chen et al. 2015). Sulforaphane was shown to exert its anticancer action in a broad number of pathways. It was shown to interfere with cancer initiation stage by modulating metabolic enzymes of both Phase I and II. Phase I metabolic enzymes initiate carcinogenesis by converting procarcinogens to carcinogens. Sulforaphane can modulate this phase I metabolism by directly interfering with P450 enzymes. Sulforaphane inhibits CYP1A1 and CYP3A4 and decease the activity of CYP3A4 (Yang et al. 1994; Maheo et al. 1997; Juge et al. 2007). Sulforaphane also modulates Phase II enzymes through antioxidant response element (ARE)-driven genes like NAD(P)H: quinone reductase 1 (NQO1), heme oxygenase 1 (HO1), and glutamate cysteine ligase (GCL) expression. Sulforaphane interacts with Kelch-like ECH-associated protein 1 (Keap1) to cause dissociation of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) from Keap1. This in turn facilitate ARE driven genes expression detoxifying carcinogens and oxidants (Talalay 2000; Brooks et al. 2001; Yoxall et al. 2005; Myzak and Dashwood 2006; Clarke et al. 2008; Kallifatidis et al. 2009). A details pathway is illustrated in Fig. 2.

The chemopreventive action of sulforaphane was found to be mediated by cell cycle arrest and apoptotic pathways (Fig. 3). Sulforaphane primarily blocks cell cycle in G2/M phase. Blocking activity in G1/S was also reported (Gamet-Payrastre et al. 2000; Wang et al. 2012). Apoptosis, which is an important regulatory mechanism for development and maintenance of homeostasis, is also modulated by sulforaphane. Sulforaphane was shown to activate intrinsic and extrinsic pathways of apoptosis. When the intrinsic or mitochondrial pathway is activated, it causes release of cytochrome C from the mitochondria, which binds to apoptosis protease activation factor-1 (Apaf-1) and finally activates caspase-9. Caspase is a family of cysteine proteases dependent pathway of apoptosis. When activated, they cause inactivation of poly(ADP-ribose) polymerase (PARP) which is a DNA repair enzyme. Extrinsic or death receptor pathway involves induction of caspase-8 and effector caspases after activation of death receptors by ligands like tumor necrosis factor-α (TNF- α) (Gamet-Payrastre et al. 2000; Keum et al. 2004; Cho et al. 2005; Karmakar et al. 2006; Choi et al. 2007; Clarke et al. 2008). Sulforaphane also induced

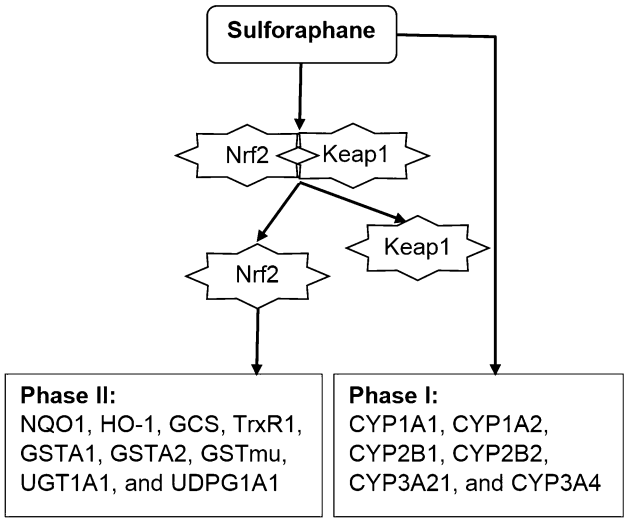

Fig. 2 Modulation of phase I and phase II metabolizing enzymes by sulforaphane.*CYP* cytochrome, *NQO1* NAD(P)H: quinone reductase, *HO1* heme oxygenase 1, *Nrf2* Nuclear factor (erythroid-derived 2)-like 2, *Keap1* Kelch-like ECH-associated protein 1, *GCS* glutamylcysteine synthetase, *GST* glutathione-S-transferase, *UGT* UDP-glucuronosyltransferases

apoptosis via induction of the proapoptotic Bcl-2 family members, generation of reactive oxygen species (ROS), and mitogen-activated protein kinases (MAPK) signal transduction (Kong et al. 2001; Kim et al. 2003; Singh et al. 2005; Sestili and Fimognari 2015). It was also reported that sulforaphane treatment increased p53 protein expression with associated increase in the protein levels of Bax (Fimognari et al. 2002). A more thorough review on the mechanisms of sulforaphane action in tumor initiation stage and tumor progression stage was reported by Myzak et al. (2006), Clarke et al. (2008), Su et al. (2018) and Liang et al. (2019).

Enhancement of anticancer activity in combination therapy

Combination therapy with two or more therapeutic agents, each having a distinct mechanism of action, is preferred over treatment with a single agent (Jia et al. 2009; Desale et al. 2015). Combination therapies target multiple cell survival pathways, which results in synergism and provides potential solution to tumor heterogeneity and drug resistance (Gottesman 2002; He et al. 2015; Yamada et al. 2016). It has been reported by many studies that sulforaphane increases the efficacy of drugs and exerts a synergistic effect by several mechanism when delivered simultaneously (Fimognari and Hrelia 2007; Wang, et al. 2009; Kallifatidis et al. 2011; Kaminski et al. 2011). A summary of the molecules showing synergism with sulforaphane is presented in Fig. 4.

Enhanced activity against pancreatic cancer

Pancreatic cancer is an aggressive malignancy. It is the 4th major cause of cancer-associated death for both women and men in the US, with slow advances in 5-year survival rate (Siegel et al. 2018). It has been reported by many studies that pancreatic cancer has been driven by cancer stem cells (CSCs), which are responsible for tumor initiation, proliferation, metastasis, and recurrence after treatment. Numerous studies also suggested the use of sulforaphane in combination with other therapeutic agents for targeting pancreatic CSCs (Olempska et al. 2007; Rausch et al. 2010; Kallifatidis

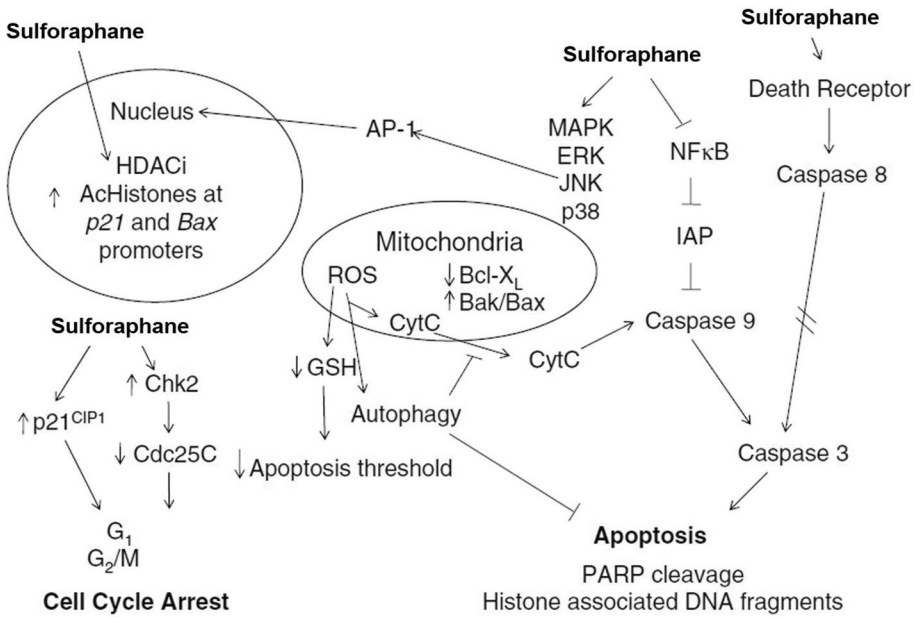

Fig. 3 Proposed ''suppression'' mechanisms of chemoprevention by sulforaphane leading to alteration in cell cycle arrest, apoptosis and/or growth inhibition (Reproduced from Clarke et al. with permission from Elsevier) *HDACi* Histone deacetylase inhibition, *Chk2* Checkpoint kinase 2, *Cdc25C* cell division cycle 25 C, *AP-1* Activator protein-1, *ROS* reactive oxygen species, *GSH* glutathione; Cyt C, *cytochrome C, MAPK* Mitogen-activated protein kinases, *ERK* extracellular-signal-regulated kinase, *JNK* c-Jun N-terminal kinases, *NFκB* nuclear factor kappa B, *IAP* inhibitor of apoptosis, *PARP* poly(ADP-ribose) polymerase

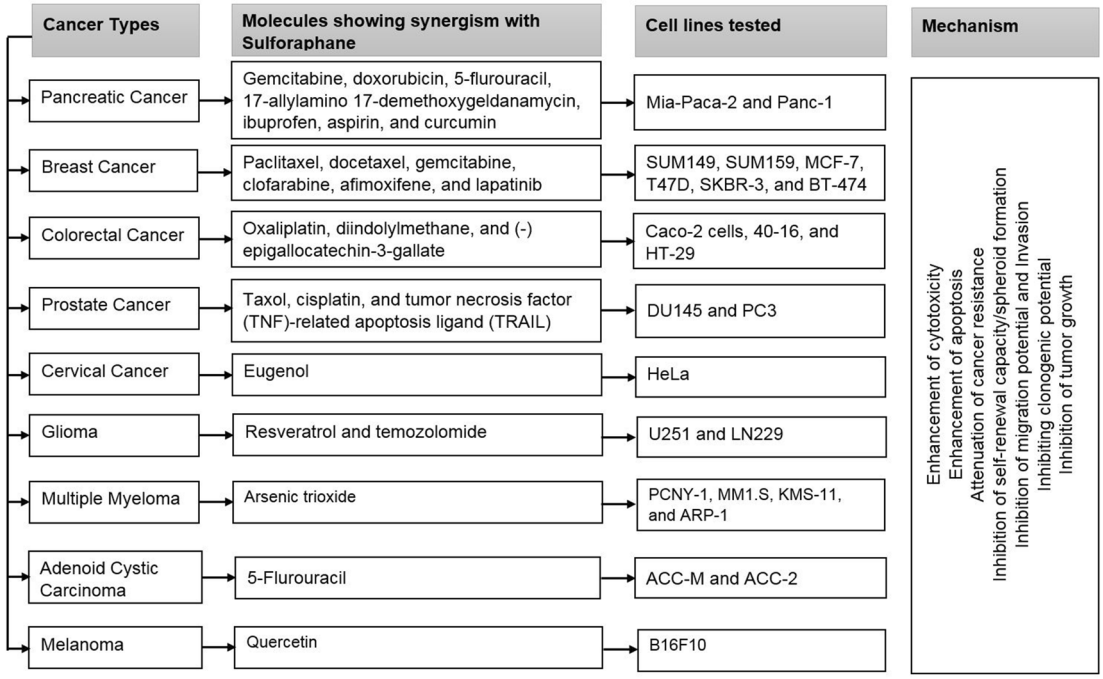

Fig. 4 Synergistic anticancer effect of sulforaphane with therapeutic molecules in different cancer types

et al. 2011). A number of studies investigated whether sulforaphane increases the activity of anticancer agents by inhibiting tumor cell proliferation, induction of apoptosis, or enhance anticancer effect against cells with high CSC features (CSC^{high})—MIA-PaCa2, and cells with low CSC features (CSC^{low})—BxPc-3, as well as Panc-1 cells (Appari et al. 2011; Thakkar et al. 2015).

Xenograft model was also used to test the efficacy of combination therapy against pancreatic cancer (Li et al. 2011; Grandhi et al. 2013).

In combined therapy, MIA-PaCa2 (CSC^{high}) cells were treated with sulforaphane (5 µM), cisplatin (CIS), gemcitabine (GEM), doxorubicin (DOX), and 5-flurouracil (5-FU) alone or in combination. After 72 h, cell viability was analyzed by MTT assay and morphological inspection. It was found that, sulforaphane increased in vitro cytotoxic effect of the anticancer drugs. Although the combination of sulforaphane with CIS, DOX, or GEM targeted only 60% of the tumor cells, combination of sulforaphane and 5-FU was most effective by targeting 80% of the cells. The combination effect of sulforaphane with GEM on clonogenic potential of CSC^{high} cells was also explored. Colony formation was reduced to 35% when cells were treated with combination of sulforaphane and GEM, whereas GEM and sulforaphane individually reduced the colony formation to 90% and 50% respectively compared to untreated controls.

Apoptosis assay also showed that GEM alone induced 30% apoptosis whereas GEM in combination with sulforaphane induced 40% apoptosis in MIA-PaCa2 cells. Similarly, sulforaphane and CIS combined treatment showed significantly enhanced apoptosis compared to each agent alone. In vivo study in nude mice with MIA-PaCa2 (CSC^{high}) cells showed similar enhanced effect of combination treatment with sulforaphane totally abolishing the growth of CSC xenografts and tumor-initiating potential (Kallifatidis et al. 2011).

A similar observation was reported for the combined treatment of sulforaphane with 17-allylamino 17-demethoxygeldanamycin (17-AAG) and ibuprofen (IBU) in Mia-Paca-2 and Panc-1 pancreatic cancer cells. Sulforaphane, at 5 µM concentration, significantly potentiated the antiproliferative effect of 17-AAG in both cell lines, with the combination index (CI) values approximately 0.62 and 0.87 for Mia Paca-2 and Panc-1 cells, respectively. In Mia Paca-2 cells, the IC_{50} of 17-AAG (0.07 µM) was more than 4-folds lower when combined with 5 µM sulforaphane than the IC_{50} of 17-AAG alone (0.31 µM). Panc-1 cells were resistant to 17-AAG with IC_{50} of approximately 11 µM. The resistance was attenuated in the presence of sulforaphane. The IC_{50} of 17-AAG was reduced to 5.47 µM when combined with 5 µM of sulforaphane. In vivo studies in athymic (nu/nu) female mice with a combination of 17-AAG (25 mg/kg, 3 times per wk) and sulforaphane (25 mg/kg, 5 times per wk) showed 70% inhibition of tumor growth, whereas sulforaphane and 17-AAG only showed 45% and 50% inhibition respectively (Li et al. 2011).

For cells treated with free-IBU (250 μM) and sulforaphane (5 μM) alone and in combination for 72 h, no significant change in cell viability was observed for treatment with single agent. However, with combination treatment at the same concentration, IBU + sulforaphane reduced the cell viability to ~ 55% showing a significant enhancement of cytotoxicity. Similar trend has been observed when IBU solid lipid nanoparticle (IBU-SLNs, 62.5 μM) and free sulforaphane (5 μM) were used for cell viability test. When treated alone with IBU-SLN and sulforaphane, there was no significant reduction in cell viability. However, combination treatment showed significant reduction of almost 80% for MIA PaCa-2 as well as Panc-1 cell lines (Thakkar et al. 2015). Synergistic effect of sulforaphane was also observed in triple combination therapy. A combination of Aspirin (ASP) with SLN (25 μM) and Curcumin (CUR) with SLN (2.5 μM), as well as free sulforaphane (5 μM) was evaluated against MIA PaCa-2 and Panc-1 cells. ASP and CUR in combination with sulforaphane reduced MIA PaCa-2 cell viability to 43.6% and Panc-1 cell viability to 48.49%, respectively when compared with individual therapy (Sutaria et al. 2012). In addition to inducing apoptosis, sulforaphane exerts its synergistic effect by the inhibition of clonogenic potential (Kallifatidis et al. 2011), inhibition of self-renewal capacity/spheroid formation (Appari et al. 2014), sensitization of CSC to cytotoxic therapy, and the inhibition of migration potential and Invasion.

Enhanced activity against breast cancer

Breast cancer is one of the most common cancers affecting the women in the US. It accounts for 30% of new cancer cases diagnosed in women (Siegel et al. 2018). Sulforaphane was shown to enhance the anticancer activity of a range of drugs against different breast cancer types, including triple negative breast cancer. Burnett et al. (Burnett et al. 2017) investigated the combination effect of sulforaphane with paclitaxel (PTX) and docetaxel (DTX) treatment on SUM149 and SUM159 breast cancer cells in vitro and in vivo. The IC_{50} of sulforaphane, PTX and DTX for SUM149 cells was 7.5 μM, 5.6 nM and 2.6 nM, respectively. For SUM159 cells, the IC_{50}s was 7.8 μM, 14 nM and 5.0 nM respectively when each agent was applied alone. Combination of a minimally cytotoxic 5 μM sulforaphane treatment with either PTX or DTX reduced the IC_{50}s to 2.2 and 1.4 nM in SUM 149 cells and 7.5 nM and 1.9 nM in SUM159 cells, respectively. In vivo studies in SUM149 injected NOD/SCID mice also showed similar effect when treated with sulforaphane and DTX. Single agent treatment with sulforaphane (50 mg/kg, daily) and DTX (10 mg/kg, weekly) reduced the tumor volume by 37.4% and 83.2% respectively, whereas when combined the tumor volume was reduced by 92.5%. It was also demonstrated that PTX and DTX induces IL-6 secretion and result in CSCs expansion in triple negative breast cancer cells. Conversely, sulforaphane preferentially eliminates CSCs by causing the inhibition of NF-kB p65 subunit translocation, downregulation of p52 and resultant downstream transcriptional activity (Burnett et al. 2017).

Enhancement of the Gemcitabine (GEM) activity by sulforaphane against MCF-7 breast cancer cells was also reported by Hussain et al. (Hussain et al. 2013). Treatment with 5 μM and 10 μM sulforaphane reduced the cell viability by 17% and 24% respectively. Treatment with 5 mM to 10 mM GEM reduced the cell viability by 34–39%. However, a combination of 5 μM sulforaphane with 5 mM and 10 mM GEM resulted in a significant decrease in cell viability by 54 and 65%, respectively, with a CI value of < 1, proving the synergistic effect. They also demonstrated that sulforaphane downregulates Bcl-2 and COX-2 to induce apoptosis and anti-inflammatory effects on MCF-7 cells, respectively (Hussain et al. 2013). Similar effects were also observed when MCF-7 cells were treated with clofarabine along with physiologically relevant sulforaphane concentration of 10 μM. It was found that sulforaphane increased cancer cell growth inhibition and apoptosis by enhancing the epigenetic effects of clofarabine at non-invasive stages of breast cancer (Lubecka-Pietruszewska et al. 2015). In addition to exerting a synergistic effect, sulforaphane also sensitizes drugs against resistant breast cancer types. Anna Pawlik et al. (2016) reported that a combination of sulforaphane with 4-hydroxytamoxifen against 4-hydroxytamoxifen-resistant T47D and MCF-7 cells, inhibited 20% more cells than sulforaphane treatment alone and was 30–50% lower than the viability of the cells when treated with 4-hydroxytamoxifen alone. The CI values were < 1 for the combination denoting the synergistic effect between the molecules. Similar activity enhancement effect was reported against HER2 overexpressing breast cancer cell lines SKBR-3 and BT-474 when sulforaphane was delivered in combination with lapatinib (Kaczynska, Swierczynska, & Herman-Antosiewicz, 2015). A synergististic anticancer activity between Withaferin A (WA) and sulforaphane was also reported. It was found that these compounds in combination inhibit cell cycle progression from S to G2 phase and down-regulates the levels of Cyclin D1 and CDK4, and pRB in MDA-MB-231 and MCF-7 breast cancer cells (Royston et al. 2018). A combination of genistein (GEN) and sulforaphane also synergistically decrease cell viability and inhibit cell cycle progression to G2 phase in MDA-MB-231 and G1 phase in MCF-7 breast cancer cell lines. This combination exerts effect by downregulating HDAC2, HDAC3, KLF4, and hTERT levels (Paul et al. 2018). An elaborated list of molecules, which was potentiated by combining with sulforaphane against breast cancer was reported elsewhere (Aumeeruddy and Mahomoodally 2019).

Enhanced activity against colorectal cancer

Colorectal cancer is the third leading cause of cancer related death in the US, accounting for 9% of new cancer cases in male and 7% in females (Siegel et al. 2018). Among the available treatment options, oxaliplatin (OX) works by disrupting DNA replication and transcription. Bettina M. Kaminski et al., reported that sulforaphane can enhance the anticancer activity of OX against the colorectal cancer in vitro. The IC_{50} of sulforaphane and OX in a 24 h-cell proliferation assay on Caco-2 cells was 26.35 and 5.58 µM, respectively. When both agents were given simultaneously, the IC_{50} value was significantly reduced and the CI value was 0.3 indicating synergism between the molecules (Kaminski et al. 2011). In another study, Gerlinde Pappa et al., investigated the combination effect of sulforaphane with 3,3′-diindolylmethane (DIM) on human colon cancer cells 40–16 (derived from a random HCT116 clone). Even though they found an antagonistic effect on cytotoxicity at lower sulforaphane and DIM concentrations; at high concentrations (> 40 µM), the combination therapy worked synergistically with a CI value less than 1. They also found that, G2/M cell-cycle arrest was strongest when 10 µM sulforaphane was combined with 10 µM DIM, which was not achievable by any compound alone (Pappa et al. 2007). Nair et al. (2008) investigated the synergistic action between sulforaphane and (-) epigallocatechin-3-gallate (EGCG) against human colon carcinoma cells HT-29 AP-1. In luciferase reporter assay, combinations of sulforaphane and EGCG significantly enhanced transcriptional activation of AP-1 reporter (46-fold with 25 µM sulforaphane and 20 µM EGCG; and 175-fold with 25 µM sulforaphane and 100 µM EGCG). This synergistic effect was confirmed by isobologram analysis. In addition to sulforaphane 25 µM + EGCG 20 µM combination, twenty-five different combinations of sulforaphane with EGCG were tested. CI values for all the combinations was between 0.325–0.7 confirming the synergistic effect between sulforaphane and EGCG. Similar activity enhancement effect of sulforaphane was also observed in vivo. A dietary administration of 1.0% dibenzoylmethane (DBM) and 600 ppm sulforaphane in Male $Apc^{Min/+}$ mouse reduced colon tumor numbers by 60% and 80%, respectively. A combination administration of 300 ppm sulforaphane and 0.5% DBM for 10 weeks completely blocked the development of colon tumor (Shen et al. 2007). The effect of sulforaphane combination with 5-FU also revealed synergistic interactions between 5-FU and sulforaphane against colon cancer cell lines Caco-2 and HT-29 (Milczarek et al. 2018).

Enhanced activity against prostate cancer

Prostate cancer is the most prominent cancer type (19%) among the male cancer population and is the second leading cause of cancer related deaths in male (Siegel et al. 2018). Kallifatidis et al. (2011) investigated the effect of sulforaphane on taxol (TAX) and CIS treatment against the DU145 prostate cancer cells, which comprises cells with CSC properties such as high proliferative, tumorigenic and invasive potential, and therapy resistance. In a 72 h MTT assay, it was found that sulforaphane potentiated both CIS and TAX in low dose (2.5 and 5 nM concentration) therapy. In case of inhibiting clonogenic potential, long term treatment with combination of TAX and sulforaphane completely abrogated clonogenicity. Likewise, sulforaphane increased apoptosis when delivered in combination with TAX (approximately 80%) and CIS (approximately 50%) compared to each agent alone, which further substantiated the activity enhancement effect of sulforaphane against prostate cancer cells.

Labsch et al. (2014) demonstrated the superiority of sulforaphane and human tumor necrosis factor (TNF)-related apoptosis ligand (TRAIL) combination treatment over single treatment against prostate cancer cell lines DU145 and PC3. Colony formation assay showed that sulforaphane reduced the clonogenic cell division to approximately 50% whereas TRAIL had minimal effect. However, a combination treatment with sulforaphane and TRAIL completely inhibited the colony formation. In an in vivo study, untreated control or in vitro-treated PC3 cells were xenotransplanted to the chorioallantois membrane (CAM) of fertilized chicken eggs. After 9 days it was found that sulforaphane reduced the tumor engraftment of the untreated cells from 78 to 43%, TRAIL reduced it to 38%, and the combination treatment to 13%. Tumor growth inhibition assay with fertilized chicken eggs showed that untreated xenograft had the tumor volume of 20 mm^3 at day 18 where the PC3 cells were transplanted on the 9th day of embryonic development and treated at day 11. Sulforaphane or TRAIL alone reduced the tumor volume to approximately 15% whereas the combination treatment of sulforaphane and TRAIL almost abolished the tumor, reducing the volume to 4 mm^3.

Enhanced activity against other cancers

Sulforaphane was shown to potentiate a range of other anticancer agents against cervical cancer, glioma/glioblastoma, bladder tumor, bronchial carcinoid tumors, lung cancer, salivary gland carcinoma, and melanoma. Hussain et al. (2012) reported the synergistic effect of sulforaphane with eugenol against HeLa cervical cancer cell line. A sublethal dose of 6.5 µM and 8 µM of sulforaphane showed 30% and 37% cell death respectively and a 200 µM and 350 µM of eugenol treatment showed 21% and 32% decrease in cell viability when treated with single agent. However, combination treatment with sulforaphane (6.5 µM) and eugenol (200 µM) showed an enhanced 55% decrease in cell

viability. Likewise, for sulforaphane + Eugenol combination at dose of 6.5 µM + 350 µM, 8 µM + 200 µM, and 8 µM + 350 µM showed significantly enhanced 67%, 60%, and 75% decrease in cell viability, respectively. The strongest synergism with a CI value of 0.7 was observed for 8 µM sulforaphane + 350 µM eugenol combination. They demonstrated that the enhanced activity was mediated by down-regulation of the COX-2, Bcl-2 and IL-β expression.

Jiang et al. (2010) demonstrated the activity enhancement effect of sulforaphane on Resveratrol (RES) therapy against Human U251 glioma cells, which are one of the most common brain tumor. In MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay with 25 µM of RES or 25 µM of sulforaphane for 24 h, they found that the cell viability was decrease to 86 and 71% of the control, respectively. Whereas, the combination of sulforaphane and RES reduced the cell viability to 59%. This enhancement effect of sulforaphane was further confirmed by Sulforhodamine B (SRB) assay. An 82 and 66% decrease in the cell viability was observed in case of single treatment by RES and sulforaphane, respectively. Combination of both resulted in a cell viability decrease to 52%, which was in alignment with MTS assay findings. Western blot analysis to measure the caspase-3 expression level showed a 7.5-fold increase compared to sulforaphane and RES treatment alone, demonstrating that sulforaphane and RES may cause apoptotic cell death through caspase-3 activation. Similarly, Lan et al. (2015) reported temozolomide-mediated apoptosis enhancement by sulforaphane when treated in combination with sulforaphane against human glioma cell U251 and LN229. They found that sulforaphane enhance the apoptosis by inhibiting miR-21 via Wnt/β-catenin.

Synergistic inhibitory effect of sulforaphane and 5-FU against salivary gland adenoid cystic carcinoma cell lines ACC-M and ACC-2 was reported by Wang et al. (2009). In MTT assay they found a moderate synergistic effect between sulforaphane and 5-FU at high effect levels. Kerr et al. (2018) reported the enhancement of anticancer activity of cisplatin when combined with sulforaphane against squamous cell carcinoma cells SCC-13 and HaCaT. An enhanced suppression of cell proliferation, stem cell spheroids formation, and migration of cells was observed in combination treatment compared to single agents alone.

A combination of allyl isothiocyanate with sulforaphane, showed synergism in inhibiting the growth of A549 lung cancer cells. Delivering this two molecules concurrently caused enhanced cell cycle arrest and apoptosis compared to the treatment by any single agent. The synergism was also augmented by production of intracellular reactive oxygen species (Rakariyatham et al. 2019). When gefitinib was combined with sulforaphane and tested against PC9GT cells, the combination decreased the cell proliferation, and inhibited the expression of SHH, SMO, GLI1, CD133 and CD44 compared to each agent alone (Wang et al. 2018).

Doudican et al. (2012) investigated the combination effect of sulforaphane and arsenic trioxide (ATO) on human multiple myeloma cell lines PCNY-1, MM1.S, KMS-11, MM1.R and ARP-1. A combination of 0.5 µM ATO and 3 µM sulforaphane showed that, with the exception of MM1.R cells, that sulforaphane synergistically enhanced the cytotoxicity of ATO with CI value of < 1 for all other cell lines. When a combination of sulforaphane with quercetin was tested in vivo with B16F10 melanoma cells tumor xenograft, an enhanced anticancer activity via decrease in MMP-9 expression was also observed compared to each agent alone (Pradhan et al. 2010). These results provided ample evidence that combining sulforaphane with existing therapies pose a potential option for improved outcome.

Enhancement of drug safety

Sulforaphane impart counter effect on chemotherapy induced toxicities to normal cells by activation of phase II enzymes and antioxidants. Doxorubicin (DOX), a highly effective anticancer agent, is known to be associated with cardiotoxicity. It induces cardiotoxic effect via oxidative stress resulting from production of free radical, and reactive oxygen species (ROS), as well as lipid aldehydes. Singh et al. (2015) showed that pretreatment of cardiomyoblast H9c2 cells with sulforaphane in a safe dose of 2.5 µM offer protection against toxicity induced by DOX treatment in vitro. Cell viability assay with 5 µg/ml DOX reduced the H9c2 cell viability to 45%, which was significantly improved to ~ 76% when treated with a combination of sulforaphane (2.5 µM) and DOX (5 µg/ml). In vivo study with wild type 129/sv mice revealed that combined treatment of DOX with sulforaphane reduced the 4-hydroxynonenal (4-HNE) protein adducts formation, improved the mitochondrial respiratory complex activities, activated the Nrf2 in hearts of treated mice, and prevented the down-regulation of antioxidant and antielectrophile enzymes GSTA4-4, SOD2, NQO1, and heme oxygenase 1 (HO-1) to provide protection against DOX-induced cardiotoxicity. Similar cardioprotective effect of sulforaphane against DOX induced cardiotoxicity was also reported by Li et al. (2015). They found that the protective effect of sulforaphane is mediated by the activation of the Keap1/Nrf2/ARE pathway, which consequently induce HO-1 (Li et al. 2015).

Similarly, when sulforaphane was tested in combination with selenium against normal colonic cell line CCD841; enhanced protection against free radical–mediated cell death was observed by activation of the Nrf2 signaling pathway and synergistic up-regulation of thioredoxin reductase–1 (TrxR-1) (Wang et al. 2015).

Sulforaphane delivery systems

Due to its activity against a broad range of cancers as well as its antioxidant and anti-inflammatory properties, plentiful attention has been given to the development of delivery systems for sulforaphane (Wu et al. 2014a, b; Manjili et al. 2016). Unfortunately, in aqueous media sulforaphane undergoes apparent first-order degradation where the rate constant increases with an increase in temperature and pH (Jin et al. 1999; Franklin et al. 2014; Wu et al. 2014a, b). For example, for every 10 °C change in temperature, the degradation rate was found to change by a factor of 3.15 in pH 4.0 solution. To address the stability of sulforaphane, several approaches, such as microencapsulation and complex formation of sulforaphane using different biopolymers have been investigated. It was found that microencapsulation of sulforaphane by spray drying utilizing hydroxypropyl-β-cyclodextrin, maltodextrin and isolated soybean protein as wall materials could increase thermal stability of sulforaphane. Microencapsulated sulforaphane was shown to be 20% more stable than non-encapsulated free sulforaphane at 90 °C for approximately 7 days (Tian et al. 2015). Complex formation of sulforaphane with hydroxypropyl-β-cyclodextrin at a 1:1 ratio by the co-precipitation method was also found to be effective in improving the stability of sulforaphane against heat, oxygen, and alkaline conditions (Wu et al. 2010). Inclusion complex of sulforaphane with α-cyclodextrin (αCD) was also explored. It was found that the sulforaphane-αCD was more stable than pure sulforaphane at room temperature (22 °C) and body temperature (37 °C). Sulforaphane-αCD showed comparable bioavailability when compared to the less stable preparation of sulforaphane (Fahey et al. 2017).

A range of microspheres and nanoparticles including co-polymer and gold core shell based nanoparticles were also explored as potential delivery vehicles for sulforaphane (Table 1). For example, water-soluble carboxymethylated chitosan (CMCS) and alginate mixed with sodium sulfate were used to fabricate sulforaphane entrapped microspheres. It was found that CMCS/alginate microspheres reduced the degradation of sulforaphane to 10% compared to 100% in case of free sulforaphane at pH 7.4 (Wang et al. 2011). Bovine serum albumin (BSA) based microspheres were also developed by spray drying, from which 50% of sulforaphane was released in about 16–18 h. Uptake studies in murine S91 and B16 melanoma cells showed a time dependent increase in uptake for both cells. In a 72 h cytotoxicity study, sulforaphane microspheres were found to be as efficacious as sulforaphane solution. However, in vivo experiment using B16 melanoma model showed that sulforaphane microspheres inhibited approximately 15% more tumor growth as compared to sulforaphane solution at week 4 post-treatment (Do et al. 2010). Similar observations were made when sulforaphane was trapped in iron oxide (magnetic)/BSA microsphere where a 13%-16% more cytotoxicity was observed for 30 µM or 50 µM treatment of sulforaphane microspheres against B16 cells when compared to sulforaphane in solution. Likewise, in vivo studies in C57BL/6 mice showed 18% more tumor growth inhibition by the microspheres when compared to the sulforaphane solution (Enriquez et al. 2013).

For the treatment of osteoarthritis, a poly (D, L-lactic-co-glycolic) acid (PLGA) based sulforaphane-PLGA microsphere was developed by freeze-drying. It was found that the sulforaphane-PLGA microsphere inhibited the expression of inflammatory markers such as ADAMTS-5, COX-2, and MMP-2 induced by lipopolysaccharide in articular chondrocytes. They were also found to delay the progression of surgically induced osteoarthritis in rats (Ko et al. 2013). To enhance the efficiency and stability of sulforaphane, gold coated iron oxide nanoparticles were also explored. Nanoparticles were furnished with thiolated polyethylene glycol-folic acid and thiolated polyethylene glycol-FITC. An increase in the cytotoxic effect was observed when MCF-7 breast cancer cells were treated with 1.5 and 3 µmol/l sulforaphane loaded nanoparticle when compared with free sulforaphane (Manjili et al. 2016). A PEGylated version of iron oxide-gold core shell nanoparticles was also investigated as a delivery system of sulforaphane alone or in combination with curcumin (CUR). The in vitro activity of sulforaphane against MCF-7 cells was increased when entrapped in the PEGylated iron oxide-gold core shell nanoparticles compared to free sulforaphane (Danafar et al. 2017a).

Sulforaphane based self-emulsifying drug delivery systems (SEDDS) were also developed to efficiently deliver sulforaphane in combination with CUR and taxanes. The SEDDS was readily soluble in water resulting in stable transparent microemulsions. When tested in vitro against MDA-MB-231 and MCF7 cancer cells by IncuCyte® live cell analysis and CellTiter-Blue® assay, taxanes/sulforaphane microemulsions showed similar activity as the commercial taxanes formulations. Additionally, when used at high concentration sulforaphane was found to potentiate the activity of taxanes (Kamal and Nazzal, 2018a, b).

Similarly, loratadine (LOR) self-microemulsifying drug delivery systems (SMEDDS) in combination with sulforaphane was developed and tested for the synergistic chemoprevention of pancreatic cancer (Desai et al. 2019). Optimum SMEDDS containing LOR-sulforaphane emulsified with tween 80 and transcutol HP resulted in emulsion with droplet size of 95 nm. When tested against pancreatic cancer cells MIA PaCa-2 and Panc-1, showed 40-fold reduction in IC_{50} concentration compared to LOR alone (Desai et al. 2019).

Sulforaphane was also loaded in nanostructured lipid carriers for oral delivery for cancer therapy (Soni et al. 2018). Lipid carrier consiting of precirol® ATO, vitamin E,

Table 1 Sulforaphane delivery systems, composition, and their outcomes

Delivery systems	Composition	Outcomes/characteristics	References
pH-sensitive microsphere	Carboxymethylated chitosan and alginate mixed with sodium sulfate, sulforaphane	Increased release of sulforaphane in the simulated colon condition, in comparison with the microspheres without sodium Enhanced stability of sulforaphane to free sulforaphane at pH 7.4	Wang et al. (2011)
Microspheres	Bovine serum albumin and R,S-sulforaphane	Time dependent increase in uptake for Murine S91 and B16 melanoma cells Comparable cytotoxicity with sulforaphane solution in in vitro cytotoxicity study Enhanced tumor inhibition in vivo compared to sulforaphane solution	Do et al. (2010)
Targeted magnetic microspheres	Bovine serum albumin, glutaraldehyde, magnetic particles consist of Iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate, R,S-sulforaphane	Enhanced cytotoxicity of sulforaphane microspheres against B16 cells to sulforaphane in solution Increased tumor growth inhibition in C57BL/6 mice by the microspheres when compared to the sulforaphane solution	Enriquez et al. (2013)
Microspheres	75:25 poly(lactic-co-glycolic acid) (PLGA), polyvinyl alcohol, sulforaphane	Inhibition of the mRNA and protein expression of COX-2, ADAMTS-5 and MMP-2 induced by LPS in articular chondrocytes Delayed the progression of surgically induced osteoarthritis in rats	Ko et al. (2013)
Gold core shell nanoparticles	Gold-coated iron oxide, Thiolated polyethylene glycol-folic acid and thiolated polyethylene glycol-FITC, D,L-sulforaphane	Increase cytotoxicity against MCF-7 breast cancer cells Induced apoptosis in MCF-7 cells	Manjili et al. (2016)
PEGylated iron oxide-gold nanoparticles	Polyethylene glycol, 2:1 ratio of ferric and ferrous chloride, $HAuCl_4.4H_2O$, D,L-sulforaphane	Enhanced cytotoxicity of sulforaphane against SKBR-3 in comparison to free sulforaphane Increase therapeutic effects by apoptosis and necrosis induction as well as inhibiting of migration in SK-BR-3 cell line	Danafar et al. (2017a)
Microemulsions	Vitamin E TPGS, transcutol HP, paclitaxel/docetaxel, D,L-sulforaphane	Sulforaphane was found to potentiate the activity of taxanes, against MDA-MB-231 and MCF7 cancer cells	Kamal and Nazzal (2018b)
Micelles	Monomethoxypoly (ethylene glycol)–poly (e-caprolactone), D,L-sulforaphane	Showed significant cytotoxicity against MCF-7 cell line Efficiently induced apoptosis in MCF-7 cell line	Danafar al. (2017b)
Micelles	Poly (caprolactone)-poly (ethylene glycol)-Poly (caprolactone) (PCL–PEG–PCL), D,L- sulforaphane	Reduced the tumor size significantly In vivo results showed that the multiple injections could prolong the circulation period and increase the therapeutic efficacy	Manjili et al. (2017)
Microcapsules	Maltodextrin/gum Arabic/carrageenan/cyclodextrin, sulforaphane	The stability of sulforaphane in spray dried microcapsules utilizing maltodextrin was greatly enhanced compared with that of free sulforaphane	Wu et al. (2014b)
Microemulsions (SMEDDS)	Tween 80 and transcutol HP, sulforaphane, LOR	Significant enhancement in the in vitro dissolution of LOR 40-fold reduction in IC50 compared to LOR alone in MIA PaCa-2 and Panc-1 7-fold and 11-fold reduction in IC_{50} compared to LOR- sulforaphane in solvent	Desai et al. (2019)
Nanostructured lipid carrier	Precirol® ATO, vitamin E, poloxamer 188, and tween 80, D,L-sulforaphane	Enhanced sulforaphane release from lipid carrier compared to suspension Increased cytotoxicity against MFC-7, SW620 and B16-F10 cancer cells 5.04-fold increase in relative oral bioavailability compared to suspension	Soni et al. (2018)

Table 1 (continued)

Delivery systems	Composition	Outcomes/characteristics	References
Liposomes	DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), sulforaphane, DOX	Liposomes containing sulforaphane and DOX exhibited synergistic interaction in MDA-MB-231 cells and additive effect in MCF-7 cells Liposomal DOX/sulforaphane combination exhibited a faster internalization rate	Mielczarek et al. (2019)

poloxamer 188, and tween 80 yielded stable emulsion with mean particle size of 145.38 nm. Sulforaphane loaded lipid carrier showed improved ex vivo gut permeation, enhanced cytotoxicity against lung, colon, melanoma cells, and five-fold enahnced oral bioavailability in rat model compared to sulforaphane solution/suspension (Soni et al. 2018). A liposome formulation consist of DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) with a diameter of 100 nm was also developed to deliver sulforaphane and DOX simultaneously and tested against breast cancer cell line MDA-MB-231 and MCF-7. A strong synergistic activity of the examined combination was observed with enhanced cellular endocytotic internalization (Mielczarek et al. 2019).

Polymeric micelles based on monomethoxypoly (ethylene glycol)–poly (e-caprolactone) (mPEG–PCL) and Poly (caprolactone)-poly (ethylene glycol)- Poly (caprolactone) (PCL–PEG–PCL) copolymer were also used for sulforaphane delivery with 86% and 87.1% encapsulation efficiency, respectively. The IC_{50} of the mPEG–PCL micelles against MCF-7 cells was found to be 14.21 µM compared to 31.2 µM for unloaded sulforaphane (Danafar et al. 2017b). Similarly, the IC_{50} was decreased to 19.15 µM when sulforaphane was loaded in PCL–PEG–PCL micelles. In vivo studies with 4T1 breast tumor bearing BALB/c mice showed a 78.5% reduction in tumor volume when treated with sulforaphane loaded PCL–PEG–PCL micelles. Free sulforaphane caused 49.5% reduction in tumor volume compared control saline group. Pharmacokinetic analysis demonstrated a 55.84 fold increase in sulforaphane bioavailability, with 3.1-fold increase in C_{max}, and 5.34 fold increase in half-life (Manjili et al. 2017).

Sulforaphane clinical trials

A number of clinical trials on sulforaphane in cancer patients were carried out (www.clinicaltrials.gov), with limited results reported in the literature. In a randomized double-blind placebo controlled trial on patients with PSA relapse after prostatectomy, a daily dose of 60 mg (340 µmol) "stabilized sulforaphane" (Prostaphane®) for six months was found to lower Log PSA slope when compared to a placebo group ($p = 0.01$) (Cipolla et al. 2014). Another study evaluated the chemopreventive effect of sulforaphane on selective biomarkers from blood and breast tissues. In a double-blinded, randomized controlled trial on patients with abnormal mammograms, who were scheduled for breast biopsy, a significant decrease in Ki-67 and HDAC3 in benign tissues was reported from patients in the sulforaphane group when compared to placebo group (Atwell et al. 2015a, b). In another study where a 200 µmoles/day of sulforaphane-rich extract was given for 20 weeks to men with prostate cancer, one subject experienced a ≥ 50% PSA decline. (Alumkal et al. 2015). In addition to completed clinical trials with available results online, several trials on the efficacy of sulforaphane on different cancers are currently underway. An extensive list of the clinical trials were summarized elsewhere (Amjad et al. 2015; Yagishita et al. 2019).

Conclusion

Since the identification of sulforaphane as an anticancer molecule with broad activity against a wide range of cancers, a good number of studies have investigated its pharmacological and delivery aspects. In this review, a brief account of sulforaphane identification, its mechanisms of anticancer action, and the findings on the activity enhancement potential of sulforaphane against several cancers when delivered with a number of anticancer agents was summarized. sulforaphane was found in numerous studies to synergize the activity of a broad range of molecules from different chemical classes. An overview of the delivery systems that have been developed to enhance sulforaphane stability and delivery has also been presented. Due to the challenges associate with the poor stability profile of sulforaphane, only a handful of delivery systems have been developed and tested to date. Since most studies on the anticancer activity of sulforaphane have been performed with sulforaphane as is, it is probable that a delivery system could have led to different outcomes. Further investigation is warranted to develop robust delivery systems to deliver sulforaphane alone or in combination with other agents to enhance the anticancer effects of sulforaphane and reduce the side effects in combination cancer therapy. This reiterates the necessity to continue investigating the promising activity of sulforaphane and to promote translation research form bench-to-bedside.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

References

Alumkal JJ, Slottke R, Schwartzman J, Cherala G, Munar M, Graff JN, Beer TM, Ryan CW, Koop DR, Gibbs A, Gao L, Flamiatos JF, Tucker E, Kleinschmidt R, Mori M (2015) A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer. Invest New Drugs 33(2):480–489. https://doi.org/10.1007/s10637-014-0189-z

Amjad AI, Parikh RA, Appleman LJ, Hahm ER, Singh K, Singh SV (2015) Broccoli-derived sulforaphane and chemoprevention of prostate cancer: from bench to bedside. Curr Pharmacol Rep 1(6):382–390

Appari M, Babu KR, Kaczorowski A, Gross W, Herr I (2014) Sulforaphane, quercetin and catechins complement each other in elimination of advanced pancreatic cancer by miR-let-7 induction and K-ras inhibition. Int J Oncol 45(4):1391–1400. https://doi.org/10.3892/ijo.2014.2539

Atwell LL, Beaver LM, Shannon J, Williams DE, Dashwood RH, Ho E (2015a) Epigenetic regulation by sulforaphane: opportunities for breast and prostate cancer chemoprevention. Curr Pharmacol Rep 1(2):102–111. https://doi.org/10.1007/s40495-014-0002-x

Atwell LL, Zhang Z, Mori M, Farris P, Vetto JT, Naik AM, Oh KY, Thuillier P, Ho E, Shannon J (2015b) Sulforaphane bioavailability and chemopreventive activity in women scheduled for breast biopsy. Cancer Prev Res (Phila) 8(12):1184–1191

Aumeeruddy MZ, Mahomoodally MF (2019) Combating breast cancer using combination therapy with 3 phytochemicals: piperine, sulforaphane, and thymoquinone. Cancer 125(10):1600–1611. https://doi.org/10.1002/cncr.32022

Brooks JD, Paton VG, Vidanes G (2001) Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol Biomarkers Prev 10(9):949–954

Burnett JP, Lim G, Li Y, Shah RB, Lim R, Paholak HJ, McDermott SP, Sun L, Tsume Y, Bai S, Wicha MS, Sun D, Zhang T (2017) Sulforaphane enhances the anticancer activity of taxanes against triple negative breast cancer by killing cancer stem cells. Cancer Lett 394:52–64. https://doi.org/10.1016/j.canlet.2017.02.023

Chen CY, Yu ZY, Chuang YS, Huang RM, Wang TC (2015) Sulforaphane attenuates EGFR signaling in NSCLC cells. J Biomed Sci 22:38. https://doi.org/10.1186/s12929-015-0139-x

Cheung KL, Kong AN (2010) Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. The AAPS Journal 12(1):87–97. https://doi.org/10.1208/s12248-009-9162-8

Cho SD, Li G, Hu H, Jiang C, Kang KS, Lee YS, Kim SH, Lu J (2005) Involvement of c-Jun N-terminal kinase in G2/M arrest and caspase-mediated apoptosis induced by sulforaphane in DU145 prostate cancer cells. Nutr Cancer 52(2):213–224. https://doi.org/10.1207/s15327914nc5202_11

Choi S, Lew KL, Xiao H, Herman-Antosiewicz A, Xiao D, Brown CK, Singh SV (2007) D,L-Sulforaphane-induced cell death in human prostate cancer cells is regulated by inhibitor of apoptosis family proteins and Apaf-1. Carcinogenesis 28(1):151–162. https://doi.org/10.1093/carcin/bgl144

Cipolla BG, Mandron E, Lefort JM, Coadou Y, Negra ED, Scodan L CL, Mottet N (2014) First double-blind placebo-controlled, multicenter, randomized trial of stabilized natural sulforaphane in men with rising PSA following radical prostatectomy. J Clin Oncol 32:5s. abstr 5032.

Clarke JD, Dashwood RH, Ho E (2008) Multi-targeted prevention of cancer by sulforaphane. Cancer Lett 269(2):291–304. https://doi.org/10.1016/j.canlet.2008.04.018

Cornblatt BS, Ye L, Dinkova-Kostova AT, Erb M, Fahey JW, Singh NK, Chen MS, Stierer T, Garrett-Mayer E, Argani P, Davidson NE, Talalay P, Kensler TW, Visvanathan K (2007) Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. Carcinogenesis 28(7):1485–1490. https://doi.org/10.1093/carcin/bgm049

Danafar H, Sharafi A, Askarlou S, Manjili HK (2017a) Preparation and characterization of pegylated iron oxide-gold nanoparticles for delivery of sulforaphane and curcumin. Drug Res (Stuttg) 67(12):698–704. https://doi.org/10.1055/s-0043-115905

Danafar H, Sharafi A, Manjili HK, Andalib S (2017b) Sulforaphane delivery using mPEG-PCL co-polymer nanoparticles to breast cancer cells. Pharm Dev Technol 22(5):642–651. https://doi.org/10.3109/10837450.2016.1146296

Desai P, Thakkar A, Ann D, Wang J, Prabhu S (2019) Loratadine self-microemulsifying drug delivery systems (SMEDDS) in combination with sulforaphane for the synergistic chemoprevention of pancreatic cancer. Drug Deliv Transl Res 9(3):641–651. https://doi.org/10.1007/s13346-019-00619-0

Desale SS, Soni KS, Romanova S, Cohen SM, Bronich TK (2015) Targeted delivery of platinum-taxane combination therapy in ovarian cancer. J Control Release 220(Pt B):651–659. https://doi.org/10.1016/j.jconrel.2015.09.007

Do DP, Pai SB, Rizvi SA, D'Souza MJ (2010) Development of sulforaphane-encapsulated microspheres for cancer epigenetic therapy. Int J Pharm 386(1–2):114–121. https://doi.org/10.1016/j.ijpharm.2009.11.009

Doudican NA, Wen SY, Mazumder A, Orlow SJ (2012) Sulforaphane synergistically enhances the cytotoxicity of arsenic trioxide in multiple myeloma cells via stress-mediated pathways. Oncol Rep 28(5):1851–1858. https://doi.org/10.3892/or.2012.1977

Enriquez GG, Rizvi SAA, D'Souza MJ, Do DP (2013) Formulation and evaluation of drug-loaded targeted magnetic microspheres for cancer therapy. Int J Nanomedicine 8:1393–1402. https://doi.org/10.2147/IJN.S43479

Fahey JW, Talalay P (1999) Antioxidant functions of sulforaphane: a potent inducer of Phase II detoxication enzymes. Food Chem Toxicol 37(9–10):973–979

Fahey JW, Wade KL, Wehage SL, Holtzclaw WD, Liu H, Talalay P, Fuchs E, Stephenson KK (2017) Stabilized sulforaphane for clinical use: Phytochemical delivery efficiency. Mol Nutr Food Res. https://doi.org/10.1002/mnfr.201600766

Fimognari C, Hrelia P (2007) Sulforaphane as a promising molecule for fighting cancer. Mutat Res 635(2–3):90–104. https://doi.org/10.1016/j.mrrev.2006.10.004

Fimognari C, Nusse M, Berti F, Iori R, Cantelli-Forti G, Hrelia P (2002) Cyclin D3 and p53 mediate sulforaphane-induced cell cycle delay and apoptosis in non-transformed human T lymphocytes. Cell Mol Life Sci 59(11):2004–2012

Franklin SJ, Dickinson SE, Karlage KL, Bowden GT, Myrdal PB (2014) Stability of sulforaphane for topical formulation. Drug Dev Ind Pharm 40(4):494–502. https://doi.org/10.3109/03639045.2013.768634

Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont MA, Chevolleau S, Gasc N, Tulliez J, Terce F (2000) Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer Res 60(5):1426–1433

Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med 53:615–627. https://doi.org/10.1146/annurev.med.53.082901.103929

Grandhi BK, Thakkar A, Wang J, Prabhu S (2013) A novel combinatorial nanotechnology-based oral chemopreventive regimen

demonstrates significant suppression of pancreatic cancer neoplastic lesions. Cancer Prev Res (Phila) 6(10):1015–1025. https://doi.org/10.1158/1940-6207.capr-13-0172

He C, Lu J, Lin W (2015) Hybrid nanoparticles for combination therapy of cancer. J Control Release 219:224–236. https://doi.org/10.1016/j.jconrel.2015.09.029

Hussain A, Mohsin J, Prabhu SA, Begum S, Nusri QA, Harish G, Javed E, Khan MA, Sharma C (2013) Sulforaphane inhibits growth of human breast cancer cells and augments the therapeutic index of the chemotherapeutic drug, gemcitabine. Asian Pac J Cancer Prev 14(10):5855–5860

Hussain A, Priyani A, Sadrieh L, Brahmbhatt K, Ahmed M, Sharma C (2012) Concurrent sulforaphane and eugenol induces differential effects on human cervical cancer cells. Integr Cancer Ther 11(2):154–165. https://doi.org/10.1177/1534735411400313

Jia J, Zhu F, Ma X, Cao Z, Cao ZW, Li Y, Li YX, Chen YZ (2009) Mechanisms of drug combinations: interaction and network perspectives. Nat Rev Drug Discov 8(2):111–128. https://doi.org/10.1038/nrd2683

Jiang H, Shang X, Wu H, Huang G, Wang Y, Al-Holou S, Gautam SC, Chopp M (2010) Combination treatment with resveratrol and sulforaphane induces apoptosis in human U251 glioma cells. Neurochem Res 35(1):152–161. https://doi.org/10.1007/s11064-009-0040-7

Jin Y, Wang M, Rosen RT, Ho CT (1999) Thermal degradation of sulforaphane in aqueous solution. J Agric Food Chem 47(8):3121–3123

Jo GH, Kim GY, Kim WJ, Park KY, Choi YH (2014) Sulforaphane induces apoptosis in T24 human urinary bladder cancer cells through a reactive oxygen species-mediated mitochondrial pathway: the involvement of endoplasmic reticulum stress and the Nrf2 signaling pathway. Int J Oncol 45(4):1497–1506. https://doi.org/10.3892/ijo.2014.2536

Juge N, Mithen RF, Traka M (2007) Molecular basis for chemoprevention by sulforaphane: a comprehensive review. Cell Mol Life Sci 64(9):1105–1127. https://doi.org/10.1007/s00018-007-6484-5

Kaczynska A, Swierczynska J, Herman-Antosiewicz A (2015) Sensitization of HER2 Positive Breast Cancer Cells to Lapatinib Using Plants-Derived Isothiocyanates. Nutr Cancer 67(6):976–986. https://doi.org/10.1080/01635581.2015.1053498

Kallifatidis G, Labsch S, Rausch V, Mattern J, Gladkich J, Moldenhauer G, Büchler MW, Salnikov AV, Herr I (2011) Sulforaphane increases drug-mediated cytotoxicity toward cancer stem-like cells of pancreas and prostate. Mol Ther 19(1):188–195. https://doi.org/10.1038/mt.2010.216

Kallifatidis G, Rausch V, Baumann B, Apel A, Beckermann BM, Groth A, Mattern J, Li Z, Kolb A, Moldenhauer G, Altevogt P, Wirth T, Werner J, Schemmer P, Büchler MW, Salnikov AV, Herr I (2009) Sulforaphane targets pancreatic tumour-initiating cells by NF-kappaB-induced antiapoptotic signalling. Gut 58(7):949–963. https://doi.org/10.1136/gut.2008.149039

Kamal MM, Nazzal S (2018a) Development of a new class of sulforaphane-enabled self-emulsifying drug delivery systems (SFN-SEDDS) by high throughput screening: a case study with curcumin. Int J Pharm 539(1–2):147–156. https://doi.org/10.1016/j.ijpharm.2018.01.045

Kamal MM, Nazzal S (2018b) Novel sulforaphane-enabled self-microemulsifying delivery systems (SFN-SMEDDS) of taxanes: formulation development and in vitro cytotoxicity against breast cancer cells. Int J Pharm 536(1):187–198. https://doi.org/10.1016/j.ijpharm.2017.11.063

Kaminski BM, Weigert A, Brune B, Schumacher M, Wenzel U, Steinhilber D, Stein J, Ulrich S (2011) Sulforaphane potentiates oxaliplatin-induced cell growth inhibition in colorectal cancer cells via induction of different modes of cell death. Cancer Chemother Pharmacol 67(5):1167–1178. https://doi.org/10.1007/s00280-010-1413-y

Karmakar S, Weinberg MS, Banik NL, Patel SJ, Ray SK (2006) Activation of multiple molecular mechanisms for apoptosis in human malignant glioblastoma T98G and U87MG cells treated with sulforaphane. Neuroscience 141(3):1265–1280. https://doi.org/10.1016/j.neuroscience.2006.04.075

Kerr C, Adhikary G, Grun D, George N, Eckert RL (2018) Combination cisplatin and sulforaphane treatment reduces proliferation, invasion, and tumor formation in epidermal squamous cell carcinoma. Mol Carcinog 57(1):3–11. https://doi.org/10.1002/mc.22714

Keum YS, Jeong WS, Kong AN (2004) Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutat Res 555(1–2):191–202. https://doi.org/10.1016/j.mrfmmm.2004.05.024

Kim BR, Hu R, Keum YS, Hebbar V, Shen G, Nair SS, Kong AN (2003) Effects of glutathione on antioxidant response element-mediated gene expression and apoptosis elicited by sulforaphane. Cancer Res 63(21):7520–7525

Ko JY, Choi YJ, Jeong GJ, Im GI (2013) Sulforaphane-PLGA microspheres for the intra-articular treatment of osteoarthritis. Biomaterials 34(21):5359–5368. https://doi.org/10.1016/j.biomaterials.2013.03.066

Kong AN, Owuor E, Yu R, Hebbar V, Chen C, Hu R, Mandlekar S (2001) Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). Drug Metab Rev 33(3–4):255–271. https://doi.org/10.1081/dmr-120000652

Labsch S, Liu L, Bauer N, Zhang Y, Aleksandrowicz E, Gladkich J, Schönsiegel F, Herr I (2014) Sulforaphane and TRAIL induce a synergistic elimination of advanced prostate cancer stem-like cells. Int J Oncol 44(5):1470–1480. https://doi.org/10.3892/ijo.2014.2335

Lan F, Pan Q, Yu H, Yue X (2015) Sulforaphane enhances temozolomide-induced apoptosis because of down-regulation of miR-21 via Wnt/beta-catenin signaling in glioblastoma. J Neurochem 134(5):811–818. https://doi.org/10.1111/jnc.13174

Liang J, Hänsch GM, Hübner K, Samstag Y (2019) Sulforaphane as anticancer agent: a double-edged sword? Tricky balance between effects on tumor cells and immune cells. Adv Biol Regul 71:79–87. https://doi.org/10.1016/j.jbior.2018.11.006

Li B, Kim DS, Yadav RK, Kim HR, Chae HJ (2015) Sulforaphane prevents doxorubicin-induced oxidative stress and cell death in rat H9c2 cells. Int J Mol Med 36(1):53–64. https://doi.org/10.3892/ijmm.2015.2199

Li SH, Fu J, Watkins DN, Srivastava RK, Shankar S (2013) Sulforaphane regulates self-renewal of pancreatic cancer stem cells through the modulation of Sonic hedgehog-GLI pathway. Mol Cell Biochem 373(1–2):217–227. https://doi.org/10.1007/s11010-012-1493-6

Li Y, Zhang T, Schwartz SJ, Sun D (2011) Sulforaphane potentiates the efficacy of 17-allylamino 17-demethoxygeldanamycin against pancreatic cancer through enhanced abrogation of Hsp90 chaperone function. Nutr Cancer 63(7):1151–1159. https://doi.org/10.1080/01635581.2011.596645

Lubecka-Pietruszewska K, Kaufman-Szymczyk A, Stefanska B, Cebula-Obrzut B, Smolewski P, Fabianowska-Majewska K (2015) Sulforaphane alone and in combination with clofarabine epigenetically regulates the expression of DNA methylation-silenced tumour suppressor genes in human breast cancer cells. J Nutrigenet Nutrigenomics 8(2):91–101. https://doi.org/10.1159/000439111

Maheo K, Morel F, Langouet S, Kramer H, Le FE, Ketterer B, Guillouzo A (1997) Inhibition of cytochromes P-450 and induction

of glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. Cancer Res 57(17):3649–3652

Manjili HK, Ma'mani L, Tavaddod S, Mashhadikhan M, Shafiee A, Naderi-Manesh H (2016) D, L-sulforaphane loaded Fe3O4@ gold core shell nanoparticles: a potential sulforaphane delivery system. PLoS ONE 11(3):e0151344. https://doi.org/10.1371/journal.pone.0151344

Manjili HK, Sharafi A, Attari E, Danafar H (2017) Pharmacokinetics and in vitro and in vivo delivery of sulforaphane by PCL-PEG-PCL copolymeric-based micelles. Artif Cells Nanomed Biotechnol 45(8):1728–1739. https://doi.org/10.1080/21691401.2017.1282501

Mielczarek L, Krug P, Mazur M, Milczarek M, Chilmonczyk Z, Wiktorska K (2019) In the triple-negative breast cancer MDA-MB-231 cell line, sulforaphane enhances the intracellular accumulation and anticancer action of doxorubicin encapsulated in liposomes. Int J Pharm 558:311–318. https://doi.org/10.1016/j.ijpharm.2019.01.008

Milczarek M, Mielczarek L, Lubelska K, Dąbrowska A, Chilmonczyk Z, Matosiuk D, Wiktorska K (2018) In vitro evaluation of sulforaphane and a natural analog as potent inducers of 5-fluorouracil anticancer activity. Molecules. https://doi.org/10.3390/molecules23113040

Moriarty RM, Naithani R, Kosmeder J, Prakash O (2006) Cancer chemopreventive activity of sulforamate derivatives. Eur J Med Chem 41(1):121–124. https://doi.org/10.1016/j.ejmech.2005.10.002

Myzak MC, Dashwood RH (2006) Chemoprotection by sulforaphane: Keep one eye beyond Keap1. Cancer Lett 233(2):208–218. https://doi.org/10.1016/j.canlet.2005.02.033

Nair S, Hebbar V, Shen G, Gopalakrishnan A, Khor TO, Yu S, Xu C, Kong AN (2008) Synergistic effects of a combination of dietary factors sulforaphane and (−) epigallocatechin-3-gallate in HT-29 AP-1 human colon carcinoma cells. Pharm Res 25(2):387–399. https://doi.org/10.1007/s11095-007-9364-7

Nakagawa K, Umeda T, Higuchi O, Tsuzuki T, Suzuki T, Miyazawa T (2006) Evaporative light-scattering analysis of sulforaphane in broccoli samples: quality of broccoli products regarding sulforaphane contents. J Agric Food Chem 54(7):2479–2483. https://doi.org/10.1021/jf051823g

Olempska M, Eisenach PA, Ammerpohl O, Ungefroren H, Fandrich F, Kalthoff H (2007) Detection of tumor stem cell markers in pancreatic carcinoma cell lines. Hepatobiliary Pancreat Dis Int 6(1):92–97

Pappa G, Strathmann J, Lowinger M, Bartsch H, Gerhauser C (2007) Quantitative combination effects between sulforaphane and 3,3'-diindolylmethane on proliferation of human colon cancer cells in vitro. Carcinogenesis 28(7):1471–1477. https://doi.org/10.1093/carcin/bgm044

Paul B, Li Y, Tollefsbol TO (2018) The Effects of Combinatorial Genistein and Sulforaphane in Breast Tumor Inhibition: Role in Epigenetic Regulation. Int J Mol Sci. https://doi.org/10.3390/ijms19061754

Pawlik A, Slominska-Wojewodzka M, Herman-Antosiewicz A (2016) Sensitization of estrogen receptor-positive breast cancer cell lines to 4-hydroxytamoxifen by isothiocyanates present in cruciferous plants. Eur J Nutr 55(3):1165–1180. https://doi.org/10.1007/s00394-015-0930-1

Posner GH, Cho CG, Green JV, Zhang Y, Talalay P (1994) Design and synthesis of bifunctional isothiocyanate analogs of sulforaphane: correlation between structure and potency as inducers of anticarcinogenic detoxication enzymes. J Med Chem 37(1):170–176

Pradhan SJ, Mishra R, Sharma P, Kundu GC (2010) Quercetin and sulforaphane in combination suppress the progression of melanoma through the down-regulation of matrix metalloproteinase-9. Exp Ther Med 1(6):915–920. https://doi.org/10.3892/etm.2010.144

Prochaska HJ, Santamaria AB, Talalay P (1992) Rapid detection of inducers of enzymes that protect against carcinogens. Proc Natl Acad Sci USA 89(6):2394–2398

Rakariyatham K, Yang X, Gao Z, Song M, Han Y, Chen X, Xiao H (2019) Synergistic chemopreventive effect of allyl isothiocyanate and sulforaphane on non-small cell lung carcinoma cells. Food Funct 10(2):893–902. https://doi.org/10.1039/c8fo01914b

Rausch V, Liu L, Kallifatidis G, Baumann B, Mattern J, Gladkich J, Wirth T, Schemmer P, Büchler MW, Zöller M, Salnikov AV, Herr I (2010) Synergistic activity of sorafenib and sulforaphane abolishes pancreatic cancer stem cell characteristics. Cancer Res 70(12):5004–5013. https://doi.org/10.1158/0008-5472.can-10-0066

Royston KJ, Paul B, Nozell S, Rajbhandari R, Tollefsbol TO (2018) Withaferin A and sulforaphane regulate breast cancer cell cycle progression through epigenetic mechanisms. Exp Cell Res 368(1):67–74. https://doi.org/10.1016/j.yexcr.2018.04.015

Sestili P, Fimognari C (2015) Cytotoxic and antitumor activity of sulforaphane: the role of reactive oxygen species. BioMed Res Int 2015:402386. https://doi.org/10.1155/2015/402386

Sharma C, Sadrieh L, Priyani A, Ahmed M, Hassan AH, Hussain A (2011) Anti-carcinogenic effects of sulforaphane in association with its apoptosis-inducing and anti-inflammatory properties in human cervical cancer cells. Cancer Epidemiol 35(3):272–278. https://doi.org/10.1016/j.canep.2010.09.008

Shen G, Khor TO, Hu R, Yu S, Nair S, Ho CT, Reddy BS, Huang MT, Newmark HL, Kong AN (2007) Chemoprevention of familial adenomatous polyposis by natural dietary compounds sulforaphane and dibenzoylmethane alone and in combination in ApcMin/+. Cancer Res 67(20):9937–9944

Siegel RL, Miller KD, Jemal A (2018) Cancer statistics 2018. CA Cancer J Clin 68(1):7–30. https://doi.org/10.3322/caac.21442

Singh P, Sharma R, McElhanon K, Allen CD, Megyesi JK, Benes H, Singh SP (2015) Sulforaphane protects the heart from doxorubicin-induced toxicity. Free Radic Biol Med 86:90–101. https://doi.org/10.1016/j.freeradbiomed.2015.05.028

Singh SV, Srivastava SK, Choi S, Lew KL, Antosiewicz J, Xiao D, Zeng Y, Watkins SC, Johnson CS, Trump DL, Lee YJ, Xiao H, Herman-Antosiewicz A (2005) Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. J Biol Chem 280(20):19911–19924. https://doi.org/10.1074/jbc.M412443200

Soni K, Rizwanullah M, Kohli K (2018) Development and optimization of sulforaphane-loaded nanostructured lipid carriers by the Box-Behnken design for improved oral efficacy against cancer: in vitro, ex vivo and in vivo assessments. Artif Cells Nanomed Biotechnol 46:15–31. https://doi.org/10.1080/21691401.2017.1408124

Su X, Jiang X, Meng L, Dong X, Shen Y, Xin Y (2018) Anticancer activity of sulforaphane: the epigenetic mechanisms and the Nrf2 signaling pathway. Oxid Med Cell Longev 2018:5438179. https://doi.org/10.1155/2018/5438179

Sutaria D, Grandhi BK, Thakkar A, Wang J, Prabhu S (2012) Chemoprevention of pancreatic cancer using solid-lipid nanoparticulate delivery of a novel aspirin, curcumin and sulforaphane drug combination regimen. Int J Oncol 41(6):2260–2268. https://doi.org/10.3892/ijo.2012.1636

Talalay P (2000) Chemoprotection against cancer by induction of phase 2 enzymes. BioFactors 12(1–4):5–11

Thakkar A, Chenreddy S, Wang J, Prabhu S (2015) Evaluation of ibuprofen loaded solid lipid nanoparticles and its combination regimens for pancreatic cancer chemoprevention. Int J Oncol 46(4):1827–1834. https://doi.org/10.3892/ijo.2015.2879

Tian G, Li Y, Yuan Q, Cheng L, Kuang P, Tang P (2015) The stability and degradation kinetics of Sulforaphene in microcapsules

based on several biopolymers via spray drying. Carbohydr Polym 122:5–10. https://doi.org/10.1016/j.carbpol.2015.01.003

Wang F, Wang W, Li J, Zhang J, Wang X, Wang M (2018) Sulforaphane reverses gefitinib tolerance in human lung cancer cells via modulation of sonic hedgehog signaling. Oncol Lett 15(1):109–114. https://doi.org/10.3892/ol.2017.7293

Wang H, Liang H, Yuan QP, Wang TX (2011) A novel pH-sensitive microsphere composed of CM-chitosan and alginate for sulforaphane delivery. Mater Sci Forum 687:539–547. https://doi.org/10.4028/www.scientific.net/MSF.687.539

Wang M, Chen S, Wang S, Sun D, Chen J, Li Y, Han W, Yang X, Gao HQ (2012) Effects of phytochemicals sulforaphane on uridine diphosphate-glucuronosyltransferase expression as well as cell-cycle arrest and apoptosis in human colon cancer Caco-2 cells. Chin J Physiol 55(2):134–144. https://doi.org/10.4077/cjp.2012.baa085

Wang XF, Wu DM, Li BX, Lu YJ, Yang BF (2009) Synergistic inhibitory effect of sulforaphane and 5-fluorouracil in high and low metastasis cell lines of salivary gland adenoid cystic carcinoma. Phytother Res 23(3):303–307. https://doi.org/10.1002/ptr.2618

Wang Y, Dacosta C, Wang W, Zhou Z, Liu M, Bao Y (2015) Synergy between sulforaphane and selenium in protection against oxidative damage in colonic CCD841 cells. Nutr Res 35(7):610–617. https://doi.org/10.1016/j.nutres.2015.05.011

Wiczk A, Hofman D, Konopa G (1823) Herman-Antosiewicz A (2012) Sulforaphane, a cruciferous vegetable-derived isothiocyanate, inhibits protein synthesis in human prostate cancer cells. Biochim Biophys Acta 8:1295–1305. https://doi.org/10.1016/j.bbamcr.2012.05.020

Wu H, Liang H, Yuan Q, Wang T, Yan X (2010) Preparation and stability investigation of the inclusion complex of sulforaphane with hydroxypropyl-β-cyclodextrin. Carbohydr Polym 82(3):613–617. https://doi.org/10.1016/j.carbpol.2010.05.020

Wu Y, Mao J, You Y, Liu S (2014a) Study on degradation kinetics of sulforaphane in broccoli extract. Food Chem 155:235–239. https://doi.org/10.1016/j.foodchem.2014.01.042

Wu Y, Zou L, Mao J, Huang J, Liu S (2014b) Stability and encapsulation efficiency of sulforaphane microencapsulated by spray drying. Carbohydr Polym 102:497–503. https://doi.org/10.1016/j.carbpol.2013.11.057

Yagishita Y, Fahey JW, Dinkova-Kostova AT, Kensler TW (2019) Broccoli or sulforaphane: is it the source or dose that matters? Molecules. https://doi.org/10.3390/molecules24193593

Yamada R, Suda H, Sadanari H, Matsubara K, Tuchida Y, Murayama T (2016) Synergistic effects by combination of ganciclovir and tricin on human cytomegalovirus replication in vitro. Antiviral Res 125:79–83. https://doi.org/10.1016/j.antiviral.2015.11.008

Yang CS, Smith TJ, Hong JY (1986s) Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: opportunities and limitations. Cancer Res 54(7):1982s–1986s

Yoxall V, Kentish P, Coldham N, Kuhnert N, Sauer MJ, Ioannides C (2005) Modulation of hepatic cytochromes P450 and phase II enzymes by dietary doses of sulforaphane in rats: implications for its chemopreventive activity. Int J Cancer 117(3):356–362. https://doi.org/10.1002/ijc.21191

Zhang Y, Talalay P, Cho CG, Posner GH (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci USA 89(6):2399–2403

Zhang Y, Tang L (2007) Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. Acta Pharmacol Sin 28(9):1343–1354. https://doi.org/10.1111/j.1745-7254.2007.00679.x