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Review Article

Mechanistic review of sulforaphane as a chemoprotective agent in bladder cancer

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Abstract: Regular consumption of cruciferous vegetables has numerous health benefits, including reduced cancer risk and improved patient outcomes. Sulforaphane (SFN) is an isothiocyanate found in cruciferous vegetables with a chemoprotective role against epithelial cancers, particularly of the bladder. Epithelial cells have several functions, including secretion, absorption, filtration, and protection from environmental insults. The specialized stratified epithelium of the bladder has direct and frequent contact with carcinogenic agents, increasing the likelihood of cancer initiation at this site. Carcinogen exposure, particularly from cigarette smoke or occupational exposure to aromatic amines, are the most significant risk factors for bladder cancer due to their ability to activate inflammatory pathways, induce free radicals, and damage DNA. SFN acts as an antioxidant by activating phase II enzymes involved in carcinogen detoxification to prevent DNA damage and inhibit tumor initiation, modulates multiple signaling pathways to inhibit tumor growth and progression, and has anti-inflammatory and immune-modulating properties to help protect against cancer. Due to these chemoprotective mechanisms, SFN has been studied as both mono- and adjuvant therapy in several bladder cancer models. Here we present a review of the effects of SFN on carcinogen-induced bladder cancer to support the inclusion of cruciferous vegetables as a chemoprotective strategy.

Keywords: Sulforaphane, isothiocyanate, bladder, cancer, carcinogen

Introduction

Bladder cancer incidence and risk factors

Bladder cancer is the sixth most common cancer in the US and is more prevalent in males than in females [1-3]. The estimated incidence of bladder cancer and related cancer deaths in the US in 2023 are 82,290 and 16,710, respectively [2]. Clinically, patients often present with painless hematuria but may also experience urinary urgency, frequency, or dysuria. Patients with a late-stage disease may present with lymphadenopathy, flank pain, and weight loss [4, 5]. Annually, 90% of bladder cancer diagnoses are urothelial carcinoma. Most cases (70%) will not be muscle-invasive at the time of diagnosis, but there is a 50-80% chance of bladder cancer recurrence within five years of diagnosis in these patients. Approximately 33% of newly diagnosed bladder cancers are muscle-invasive, and 4% present as distant metastatic disease [5, 6]. The five-year overall survival rate for non-muscle invasive bladder cancer is 77%. However, patients with distant metastases have a five-year survival rate as low as 6% [7].

The most significant risk factor for the development of bladder cancer is a history of cigarette smoking, which is responsible for approximately 50% of bladder cancer cases in both men and women. Those who smoke have a three-times greater chance of being diagnosed with bladder cancer than those who do not smoke [8, 9]. In older male populations, a 65+ pack-year history of smoking leads to a relative risk of 3.6 for bladder cancer compared to neversmokers (95% confidence interval [CI] = 2.2-4.6) [10]. The increased risk may, in part, be explained by increased DNA damage caused by smoking. For instance, DNA adducts, particularly 4-aminobiphenyl (cigarette smoke carcinogen) DNA adducts, are higher in bladder cancers of smokers than in non-smokers [11]. Other well-documented risk factors for bladder cancer include schistosomiasis, prior pelvic radiation, chronic bladder inflammation, and exposure to certain medications and occupational toxins, including arsenic, aromatic amines, and aniline dyes [8, 12, 13].

Isothiocyanate metabolism and bioavailability

Phytochemicals are plant-produced compounds that often serve as plant defense mechanisms and have been used for medicinal purposes for hundreds of years [14-17]. In recent decades, certain phytochemicals have emerged as potential or active anti-cancer therapies [18-21].

Isothiocyanates are active plant phytochemicals derived from inactive precursors, glucosinolates, which are uniquely present in cruciferous vegetables such as cabbage, kale, brussels sprouts, and broccoli [22, 23]. Cruciferous vegetables of the *Brassicaceae* family are believed to have originated in Europe several thousand years ago [24]. They are rich in vitamins and minerals, including folic acid, vitamin A, iron, calcium, and zinc [25]. This family of vegetables harbor varying levels of glucosinolates, which contain sulfur groups that give cruciferous vegetables their characteristic pungent taste and smell [26]. The metabolism of glucosinolates begins with plant damage, such as chewing or chopping, which activates plant-derived myrosinase enzymes that convert inactive glucosinolates into isothiocyanates, thiocyanates, nitriles, goitrin, and epithionitriles. Myrosinase enzymes can also be produced in varied quantities by gut microbiota, which can assist in glucosinolate metabolism in the colon [26-28]. Isothiocyanates are believed to be important inducers of phase II detoxification enzymes and may play a role in chemoprevention [29]. Their overall metabolism and bioavailability depend on multiple factors, including mode of consumption, stomach acidity, gut microbiome, and genetic makeup [30, 31].

Once produced, isothiocyanates undergo a series of metabolic reactions, including conjugation with glutathione by glutathione-S-transferases (GSTs) [32, 33]. GSTs are phase II detoxification enzymes that neutralize endogenous and exogenous compounds and help to reduce oxidative stress. In humans, there are two super-families of GSTs, microsomal and cytosolic. Cytosolic GSTs are coded for by at least 16 genes [34]. GSTs, particularly the cytoplasmic enzymes GSTM1 and GSTP1, play an important role in isothiocyanate metabolism. GSTM1 is highly expressed in cells of the liver, which may further support its role in metabolism of SFN after oral consumption [30, 35].

A variety of polymorphisms have been described in the GST enzymes, particularly of GSTM1 and GSTT1 [36, 37]. While the overall frequencies of homozygous GSTM1- and GSTT1-null genotypes vary depending on racial and/or ethnic group, polymorphisms in GST enzymes exist across all populations [37]. For instance, GSTM1-null mutations affect up to 53% of all racial/ethnic groups while GSTT1-null mutations affect up to 21% of Caucasian, 64% of Asian populations, and 45% of African populations [35, 38]. These mutations lead to a lack of a functional protein product, which may alter the metabolism and efficacy of isothiocyanates, particularly SFN, as chemopreventive agents [35, 39, 40].

GSTM1 mutations are perhaps the most well studied of the GST enzyme polymorphisms. In mice, knocking out GSTM1 leads to increased inflammation and oxidative stress [41]. In humans, epidemiologic studies suggest that individuals in the United States with active GSTM1 may gain a greater degree of protection from the consumption of cruciferous vegetables than those who are GSTM1-null [35]. However, epidemiologic studies in Asia suggest that GSTM1- or GSTT1-null individuals derive a higher degree of cancer protection from crucifer consumption than those who are GSTM1- and GSTT1-positive [42, 43]. The differences in the predominant type of cruciferous vegetables consumed by Asian populations (Chinese cabbage) versus American populations (broccoli) may be one reason for these conflicting results [43]. Alternatively, GSTM1 metabolism of SFN may increase its secretion, and those with GSTM1-null mutations may ultimately have reduced SFN secretion, and therefore increased circulating levels of the anti-carcinogenic compound to combat cancer [44]. Finally, additional enzymes of the mercapturic acid pathway are required for isothiocyanate metabolism and differing levels of enzyme activity among individuals may also play a role in SFN activity and cancer prevention [44].

Individuals with GSTM1-null mutations may also have compensatory mechanisms for the loss of GSTM1 activity. Work done by Gasper, et al. indicated that GSTM1-null individuals metabolize SFN more rapidly within the first six hours after consumption, and excrete more urinary SFN metabolites within a 24-hour period, compared to individuals who have functional GSTM1 proteins [35]. Additional investigations indicated that the half-life of SFN is greater in those with GSTM1-null mutations, but that the type and quantity of urinary SFN metabolites were similar to those who were GSTM1-positive [45]. In respiratory epithelial cells with GSTM1-null mutations, SFN treatment led to a dose-dependent increase in overall GST activity, believed to be a compensatory mechanism of SFN metabolism [46]. Furthermore, *in vitro* analysis of GSTM1-null human lymphocytes showed that GSTM2 expression nearly doubled with SFN treatment, and GSTM2 also metabolized SFN at a comparable rate to function GSTM1 protein [47].

Anticancer mechanisms of SFN

SFN is a bioactive compound with both antioxidant and anti-inflammatory properties. SFN induces cancer cell apoptosis and cell cycle arrest while also activating enzymes responsible for detoxifying carcinogenic compounds [48, 49]. SFN also improves the efficacy of certain traditional chemotherapeutic regimens [50, 51]. SFN acts as a chemoprotective agent through various mechanisms, including the activation of phase II enzymes involved in carcinogen detoxification to prevent DNA damage and inhibit tumor initiation, as well as the regulation of multiple signaling pathways to inhibit tumor growth and progression. SFN also possesses immune-modulating properties that help to protect against cancer [48, 49, 52]. Such functions, along with the selective toxicity towards transformed cells, make SFN a potential candidate for the treatment and prevention of several types of cancer, especially epithelial cancers associated with high carcinogen exposure, like bladder cancer. Each of the anti-cancer mechanisms of SFN are reviewed below.

Antioxidant properties of SFN

Many of the most notable effects of SFN are mediated by its electrophilic properties and ability to activate phase II detoxification enzymes. The best-established mechanism is via protein nuclear factor erythroid 2-related factor-2 (Nrf2) activation. Under normal conditions, Nrf2 is bound by its inhibitor, Kelch-like ECH-associated protein 1 (Keap1). SFN inactivates Keap1, allowing Nrf2 to translocate to the nucleus and activate specific antioxidant response elements (ARE) [53-55]. A lesser established mechanism proposed by Li, et al. is that SFN induces mild increases in reactive oxygen species (ROS), leading to transcription factor EB (TFEB) activation. TFEB plays a role in activating antioxidant response elements and regulating cellular autophagy, ultimately reducing overall oxidative stress. TFEB also acts as a potential arm of protein Nrf2 activation [55].

Nrf2 is an important leucine zipper transcription factor that assists in drug metabolism by inducing antioxidant response element-mediated expression of phase II detoxification enzymes, including NAD(P)H: quinone oxidoreductase and GSTs [56-58]. Under homeostatic conditions, Keap1 binds to and inhibits Nrf2, which is eventually ubiquitinated and degraded [59, 60]. Accumulation of ROS and electrophiles, due to SFN or other mechanisms, activates Nrf2 by modification of cysteine residues on Keap1, which weakens the association between Keap1 and Nrf2, allowing Nrf2 to disassociate from Keap1 and translocate to the cell nucleus [60, 61]. Once in the nucleus, Nrf2 proteins bind ARE to stimulate the expression of phase II enzymes (N-acetyltransferase and glutathione S-transferase), which play crucial roles in detoxifying xenobiotics [61, 62].

While phase II enzymes typically detoxify carcinogenic compounds, phase I enzymes generally activate xenobiotic compounds into their carcinogenic metabolites rather than inactivate them into neutral compounds [63-65]. In addition to activating phase II enzymes, SFN also decreases the expression of phase I enzymes,

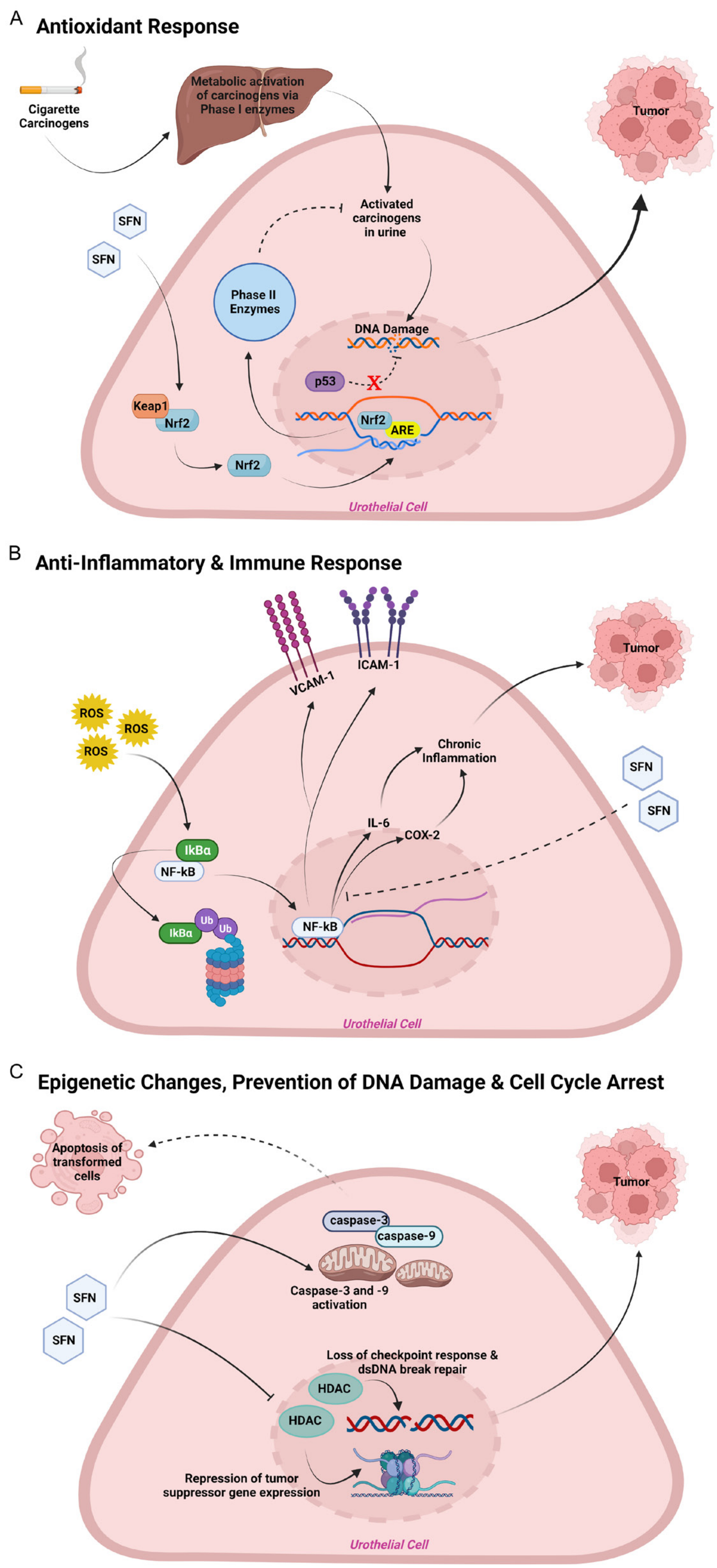

Figure 1. Chemoprotective effects of SFN. A. SFN mediates antioxidant response by Nrf2 activation of phase II enzymes with subsequent repair of tobacco carcinogen-induced DNA damage in the transitional epithelium. B. SFN induces anti-inflammatory and immune responses by inhibiting the NF-κB pathway in epithelial cells. C. SFN induces epigenetic changes, cell cycle arrest, and apoptosis of transformed epithelial cells. Figure was created with BioRender.

especially cytochrome P450 isoenzymes 2E1 (CYP2E1) and 3A4 in human hepatocytes [66, 67]. Both phase I and phase II xenobiotic metabolism occur predominantly in the liver [68].

Activating Nrf2 with a subsequent increase of phase II enzyme expression is an essential chemoprotective mechanism of SFN in the bladder (**Figure 1A**). An example of this was previously recognized in human hepatocyte and hepatoma (HepG2) cell lines wherein SFN treatment increased Nrf2 and, therefore, glutathione levels [69]. Low doses of SFN (less than or equal to 5 μM) assisted in protecting both cell lines against hydrogen-peroxide-induced damage by activating the Nrf2/glutathione detoxification system. Cancer cells treated with SFN showed higher catalase levels, heme oxygenase 1, and NAD(P):quinone oxidoreductase-1 detoxification enzymes [69]. In cultured prostate cancer cells and prostatic adenocarcinomas in murine models, SFN treatment led to increased Nrf2 levels, with a subsequent decrease in ROS caused by androgen deprivation. Furthermore, Liu, et al. revealed that SFN treatment sensitized prostate cancer cells to radiation therapy via Nrf2 upregulation [70]. By reducing oxidative stress, SFN may play a role in preventing the DNA damage, mutation, and inflammation that contribute to carcinogenesis.

Anti-inflammatory properties of SFN

Chronic inflammation is a contributing factor to the emergence of various cancer types. The relationships between pro- and anti-inflammatory cytokines, as well as inflammatory cells and their cytokine and chemokine milieus, play significant roles in the induction and progression of cancer [71, 72]. High levels of ROS also create favorable environments for inflammation, tumor initiation, and proliferation through the oxidation of critical molecular structures, including lipids, proteins, and nucleic acids [73]. Through its action on the Keap1/Nrf2 pathway and induction of ROS detoxifying enzymes, SFN may mitigate inflammation and inhibit tumor cell progression (**Figure 1B**) [73-75].

ROS also generate inflammation by increasing the levels of tumor necrosis factor-alpha (TNF-α), which in turn increases the expression of the transcription factor Nuclear Factor Kappa B (NF-κβ) [76]. NF-κβ promotes oxidative stress and can induce inflammation if homeostatic conditions are not restored [73]. The inflammatory molecules released in response to NF-κβ include interleukin-6 (IL-6), interleukin-1β (IL-1β), and interferon-gamma (INF-γ), in addition to cyclooxygenase-2 (COX-2), vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1), all of which favor inflammation [77-80]. Alternatively, SFN treatment inhibits the release of NF-κβ from the inhibitory molecule, I-kB, and can prevent NF-κβ from binding to DNA, thereby reducing the expression of COX-2 and other inflammatory mediators [81-84]. Similarly, SFN treatment has also been found to decrease the cellular release of IL-1β, IL-2, IL-6, and IL-10, reduce the expression of VCAM-1 and ICAM-1 on endothelial cells exposed to lipopolysaccharide via downregulation of NF-κβ, as well as increase cell autophagy and apoptosis of transformed cells [76, 79, 85, 86].

In addition to altering cytokine expression, SFN treatment modulates the function of several innate and adaptive immune cell types. For instance, SFN was previously found to regulate the antibody-dependent cellular cytotoxicity response of natural killer cells, leading to the destruction of recognized cancer cells [87]. SFN also plays an immunomodulatory role in certain inflammatory conditions by polarizing macrophages to an alternatively activated/M2 phenotype rather than a classically activated/M1 phenotype [88-90]. The M1 subtype is associated with a pro-inflammatory response characterized by increased inflammatory cytokines and ROS expression. Conversely, macrophages of the M2 subtype are associated with the production of anti-inflammatory cytokines and the activation of pathways that lead to antioxidant production [89, 91, 92].

Furthermore, SFN can reduce inflammation by inhibiting activated, non-transformed human T cells, especially TH17 cells, through increasing intracellular ROS levels, decreasing glutathione levels, and reducing the expression of RORgt, IL-17, and IL-22. The T cell activation markers CD25 and CD69 and the activation factor IL-2 are also decreased with SFN treatment compared to untreated controls [75]. Recently, Shen et al. discovered that SFN treatment potentiates the activities of chimeric antigen receptor T (CAR-T) cells *in vitro* and *in vivo*. CAR-T cells treated with SFN secreted more IFN-γ, perforin, and granzymes, and reduced CAR-T expression of programmed cell death 1 (PD-1), which binds programmed cell death ligand 1 (PD-L1) on tumor cells and promotes CAR-T exhaustion. In xenograft models, CAR-T cells in mice given SFN showed reduced tumor progression and prolonged survival [93]. Through the modulation of T cell activity, SFN may decrease T cell-mediated inflammation, prevent autoimmunity and potential carcinogenesis, and promote the death of transformed cells.

DNA modulation by SFN

In addition to its effects on antioxidant responses and inflammation, SFN alters cellular epigenetics by regulating histone deacetyltransferases (HDACs), which remove acetyl groups from histones, causing the repression of gene transcription (**Figure 1C**). The effects of SFN on HDACs have been shown in multiple models, including human colon and prostate cell lines, prostate cancer xenografts, and murine peripheral mononuclear cells [94]. Increased HDAC expression is a mechanism used by several cancer types to repress the expression of tumor suppressor genes and allow continued cell cycling and proliferation [95, 96]. HDACs also play a role in the DNA damage response at cell cycle checkpoints and during the processing of double-stranded DNA breaks [97]. SFN was previously shown to inhibit HDACs competitively and protect against carcinogenesis [98-101]. For instance, in LNCaP and PC3 prostate cancer cell lines, SFN-induced HDAC inhibition led to increased levels of the tumor suppressor p21 with subsequent cell cycle arrest and increased Bax levels, which induced cancer cell apoptosis [94, 102]. Furthermore, cell cycle analyses have shown that SFN inhibits cell cycle progression, particularly in the sub-G1 and G2/M phases, with

Table 1. Anti-cancer mechanisms of sulforaphane and corresponding references

Mechanism of Action	Sources
Induction of Nrf2	Kensler et al. 2013 [49]; Dinkova-Kostova et al. 2017 [54]; Subedi et al. 2019 [85]; Wang et al. 2018 [76]; Liu et al. 2019 [69]; Jo et al. 2014 [160]
Increased ROS	Liang et al. 2018 [75]; Sing et al. 2005 [107]; Jo et al. 2014 [160]
Cell cycle arrest	Wang et al. 2015 [48]; Cheng et al. 2016 [103]; Zuryn et al. 2016 [104]
Induction of apoptosis	Wang et al. 2015 [48]; Sharma et al. 2011 [173]; Hahm and Singh 2010 [86]; Sing et al. 2005 [107]; Jo et al. 2015 [160]
Enhanced anti-cancer drug effects	Calcabrini et al. 2020 [168]; Kerr et al. 2018 [167]
Micro-RNA activation	Wang et al. 2016 [50]
Enhanced immune cell function	Thejass and Kuttan 2006 [87]
Suppression of inflammation	Ali et al. 2020 [89]; Pal and Konkimalla 2016 [88]; Heiss et al. 2001 [82]; Shan et al. 2012 [79]; Sharma et al. 2011 [173]; Subedi et al. 2019 [85]; Hahm and Singh 2010 [86]
Downregulation of STAT3	Hahm and Singh 2010 [86]; Subedi et al. 2019 [85]
COX-2 suppression	Woo and Kwon 2007 [84]; Sharma et al. 2011 [173]
Cytochrome P450 inhibition	Maheo et al. 1997 [66]; Barcelo et al. 1996 [67]
Reduction of PD-1 and PD-1L expression	Shen et al. 2021 [93]
HDAC inhibition	Myzak et al. 2007 [94]; Jiang et al. 2016 [101]
Inhibition of DNA adduct formation	Singletary and MacDonald 2000 [110]; Bacon et al. 2003 [185]; Ding et al. 2010 [158]
Reduced AP-1 expression	Dickinson et al. 2009 [186]
Altered DNA methylation profiles	Li et al. 2020 [187]
Reduced polycomb group [170] gene expression	Balasubramanian et al. 2011 [170]

associated reductions in cyclins B1 and D1, along with increased tumor suppressor proteins p21 and p53 activity [103-105].

The mild increase in ROS generated by SFN may also help disrupt mitochondrial membranes and cause the release of cytochrome c with subsequent apoptosis of cancer cells [106, 107]. In HeLa cells and HepG2 cells, SFN induces the formation of apoptotic bodies and causes cell cycle arrest in the sub-G1 phase. SFN also increases Bax expression and downregulates Bcl-2 and Bcl-XL, further evidence that SFN induces apoptosis in transformed cells [108]. Additionally, in a study on SFN-induced apoptosis in prostate cancer cells, SFN led to the activation of caspase-3 and caspase-9, triggering apoptosis in tumor cells [107].

By inhibiting phase I enzymes and activating phase II detoxification enzymes, SFN may reduce the activation of carcinogens and ROS that interact with cellular DNA, thus preventing DNA mutations or reducing the mutation burden [109, 110]. Some research suggests that SFN increases DNA damage and prevents double-stranded DNA break repair, leading to cell cycle arrest and apoptosis [111-113]. However, others report a decrease in DNA mutation burdens. For example, in HepG2 hepatoma cells, SFN exposure for three hours reduced DNA adduct levels by 66% [114]. Collectively, these mechanisms help prevent tumor initiation and progression (see **Table 1** for a summary of mechanisms and corresponding references).

Origin of bladder cancer

The primary function of the bladder is to store urine until it is ready to be excreted [115]. The bladder wall is composed of four layers. From the outside to the inside, there is the adventitia, the muscular layer, the submucosal layer, and finally, the mucosal layer, or urothelium [116, 117]. The urothelium is a layer of transitional epithelium consisting of basal cells, intermediate cells, and a superficial layer of umbrella cells that line the urinary bladder cavity and act as an impermeability layer and are held together by tight junctions [118]. Umbrella cells change morphology depending on whether the bladder is full of urine or empty. These cells become flattened and elongated when the urinary bladder is full and, conversely, are taller and more cuboidal once the bladder is emptied [119]. When the bladder is relaxed, it consists of five to seven layers of cells, but when it fills with urine and is distended, the urothelium will become two to three layers [120]. Most bladder cancers begin in the transitional epithelium and can advance to deeper layers with cancer progression [121].

Carcinogen-induced field cancerization

Field cancerization

Field cancerization in which cancers develop in epithelia exposed to environmental carcinogens is well-documented, and histologically detectable precancerous changes are present throughout large sections of the carcinogen-exposed surface [122, 123]. Furthermore, evidence of clinically detectable field cancerization in histologically normal epithelia is associated with precancerous changes in the form of mutation-harboring cell groups. These clonal mutations are thought to be the first detectable manifestations of the multi-hit carcinogenic process [124]. In carcinogen-induced cancers, damage resulting in cancer formation predates cancer by decades [124]. For example, driver mutations can be detected in hematological malignancies approximately ten years before the clinical diagnosis [125]. Bladder cancer often develops multifocally, and superficial bladder cancer has high recurrence rates. One possible explanation for these phenomena is field cancerization, wherein carcinogen exposure causes DNA mutations at independent locations in the urothelium [126, 127]. This is supported by studies showing genetically-dissimilar primary and recurrent tumors [128].

Chemical carcinogen-induced bladder cancer

Smoking is the greatest risk factor for bladder cancer and contributes to approximately 50% of diagnosed cases [129-131]. In patients with a history of cigarette smoking, bladder cancers generally have higher *HER2* amplification mutations that help drive the cancers, whereas those who do not smoke more commonly have *PIK3CA* mutations [132]. Mutations in the genes *TP53, CDKN2B,* and *TERT* are common in both smokers and non-smokers [133-137].

In addition to smoking, occupational exposure to certain carcinogens, such as aromatic amines, fossil fuels, aniline dyes, or certain metals like aluminum, account for approximately 20% of bladder cancer cases [12, 138, 139]. Certain genetic factors can also contribute to developing bladder cancer. For instance, N-acetyltransferase and glutathione S-transferase gene polymorphisms can affect the abilities of these enzymes to detoxify certain carcinogens that may contribute to bladder cancer [140].

Even with early treatment, there is a significant likelihood of bladder cancer recurrence, and those with the invasive disease face poorer outcomes, even with aggressive treatment. Due to continued poor outcomes for those with bladder cancer, there is a need for new interventions to protect against and treat this disease. SFN is a possible intervention that is well tolerated and has no significant toxicities [141, 142].

Smoking carcinogen-induced epithelial cancers

Each year, smoking contributes to 180,000 cancer-related deaths in the US [143]. Cigarette smoke contains over 70 carcinogens that increase cancer risks [144]. While many of the chemicals in cigarette smoke are carcinogenic, many others are converted into carcinogenic compounds by cytochrome P450 enzymes [145]. These carcinogens and their metabolites can cause DNA adducts, contributing to tumorigenesis if not detoxified into harmless compounds, and have been shown to induce soluble factor profiles consistent with NF-κβ expression, including TNF-α, IL-1β, metalloproteases, and leukocyte-stimulating factors, among others, in mice [143, 146, 147].

Bladder cancer prevention with SFN

SFN in bladder cancer

Early evidence indicates a potential role for isothiocyanates, particularly SFN, in the chemoprevention of bladder cancer. Men who regularly consumed cruciferous vegetables were found to have a lower relative risk (0.49) for bladder cancer than men who did not consume cruciferous vegetables (P = 0.0082). This equated to a 51% risk reduction for those who consumed more than five servings of cruciferous vegetables weekly compared to those who consumed one serving or less [10, 148]. Another retrospective case-control study that analyzed isothiocyanate intake and its effects on bladder cancer risk discovered that increased isothiocyanate intake was associated with a 29% decreased risk of bladder cancer in past and current smokers and older individuals [149]. Increased cruciferous vegetable intake may also improve survival outcomes in patients with bladder cancer [150]. Raw cruciferous vegetables appear to provide the greatest risk reduction, most likely due to the higher yield of isothiocyanates [151, 152].

SFN and other isothiocyanates are secreted predominantly in the urine, further supporting the potential role of SFN in preventing bladder carcinogenesis [152, 153]. Shortly after dosing rats with broccoli sprout extract, Munday et al. observed that urinary isothiocyanate levels were 2-3 times greater in urine than in plasma [154]. Similarly, broccoli sprout consumption in humans led to isothiocyanate levels that were 50 times greater in urine than in plasma eight hours after ingestion [155]. These studies indicate that isothiocyanates are concentrated in the urine and thus contact the bladder epithelium, the primary site for bladder cancer initiation.

In N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)-induced bladder cancer models, Nrf2 regulates carcinogen detoxification genes and interacts with the p53 protein to further aid in chemoprevention. Increased tumor incidence and invasion were observed in Nrf2 null mice treated with BBN [156, 157]. SFN inhibits DNA damage in murine bladder epithelium exposed to the cigarette smoke carcinogen 4-aminobiphenyl by activating Nrf2 and downstream phase II enzymes [158]. SFN also inhibits the ubiquitination and breakdown of Nrf2, thereby allowing for increased Nrf2 nuclear translocation and anti-cancer gene induction [159].

Bladder cancer cell culture studies have shown that SFN decreases cell viability and initiates apoptosis. In T24 urinary bladder cancer cells, SFN reduces cell proliferation and leads to chromatin clumping and fragmentation, increased cytochrome c release, caspase-9 and caspase-3 activation, and subsequent T24 cell apoptosis [160]. SFN treatment of T24 cells increased ROS generation, inducing mitochondrial dysfunction and cellular apoptosis. The ROS allowed for Nrf2 release from Keap1, as measured by the induction of heme oxygenase-1. Moreover, SFN treatment increased the endoplasmic reticulum stress response pathway, further contributing to T24 cell apoptosis [160].

In UM-UC-3 bladder cancer cells, sulforaphane also induces DNA fragmentation, caspase-9, and caspase-3 cleavage, mitochondrial damage, and subsequent cellular apoptosis, in addition to G2-M cell cycle arrest *in vitro* [161, 162]. In the UM-UC-3 murine xenograft model, mice treated with SFN showed a tumor volume reduction of 67%, compared to control tumor volume 15 days after initial treatment. At 24 days of treatment, the average tumor volume was still 36% less in SFN-treated mice than in control mice. Interestingly, the tumors in mice treated with SFN decreased angiogenesis compared control tumors and had increased immune cell infiltration. There was also more than a 5-fold increase in the number of apoptotic cells in tumors of SFN-treated mice compared to tumors of control mice [163].

In rats fed broccoli sprout extract with high levels of SFN before and during exposure to BBN, there was decreased bladder cancer occurrence and reduced tumor burden compared to controls given BBN alone. Importantly, no negative impacts were observed in the bladders of rats given SFN alone, indicating not only the potential efficacy of SFN in bladder cancer chemoprevention, but also the safety of SFN treatment [154, 164].

Finally, the chemoprotective mechanisms of SFN may play an enhanced role in treating bladder cancer when used in combination with traditional chemotherapeutic drugs and may even prolong the time to therapy resistance. The chemotherapeutic agent, Everolimus, is an mTOR inhibitor and was shown to have some anti-tumor activity in patients with urothelial carcinoma [165]. Justin et al. showed that when combined with Everolimus, SFN improved therapeutic effects by reducing RT112 cancer cell chemotaxis and invasion. There was increased cancer cell therapy resistance, invasion, and migration of tumors treated with Everolimus alone [166]. SFN has also shown promise in combination with cisplatin, doxorubicin, and CAR-T cell therapy and may even help mitigate some of the toxic side effects of certain chemotherapeutics [93, 167, 168].

Discussion

SFN is an isothiocyanate found in cruciferous vegetables that have shown chemoprotective effects in various cancer models, including those of the bladder [158, 160, 163, 169, 170]. SFN induces phase II detoxification enzymes through the activation and nuclear accumulation of Nrf2 while also decreasing phase I enzyme activation, particularly cytochrome P450 isoenzymes, which can convert many compounds into carcinogenic metabolites [60, 62, 66, 67, 171]. In the urothelium, SFN also acts to decrease inflammation through the detoxification of ROS, inhibition of NF-κβ and COX-2 expression, and modulation of inflammatory cytokines, including IL-1β, IL-2, IL-6, and IFN-γ [74, 75, 82-87, 172-174]. Finally, SFN reduces DNA damage through HDAC regulation, the induction of cell cycle arrest, and the apoptosis of transformed cells [94, 98-101, 103, 104, 107]. SFN reduces tumor cell growth and invasion through the inactivation of sulfatase-2, a key component in the Wnt and fibroblast growth factor signaling pathways in many human cancers [175]. Finally, SFN activates chemoprotective mechanisms on its own and acts synergistically with some traditional chemotherapeutics to reduce tumor burden and limit the toxic side effects of traditional chemotherapy [166-168].

Besides its favorable biological activities, SFN can play a vital role in preventing and treating bladder cancer due to the active excretion and concentration in urine after isothiocyanate consumption [153-155]. Accordingly, there is compelling evidence that SFN inhibits urothelial cell DNA damage induced by cigarette carcinogens and reduces overall bladder cancer risk in those who regularly consume cruciferous vegetables [10, 148, 158]. SFN-induced Nrf2 activation has also been found to detoxify certain carcinogens from cigarette smoke and upregulate the expression of p53. SFN also induces cell cycle arrest and apoptosis in transformed bladder epithelium [156, 157, 159-161]. Taken together, these studies provide strong evidence for the chemoprotective nature of SFN in various human epithelial cancers, including those of the bladder.

Future studies

Increased cruciferous vegetable intake has previously been associated with a decreased overall cancer risk [176]. Inverse associations between cruciferous vegetable intake, especially broccoli, and bladder cancer risk, as well as bladder cancer mortality have been reported in epidemiological studies [177, 178]. The potent chemopreventive and chemotherapeutic effects of SFN have been well documented in cell culture and animal models of bladder cancer [158, 179, 180]. However, to translate these findings to the bedside, it is essential to validate these findings in prospective cohort studies and to further investigate the effects of SFN-rich diets in humans, especially those with elevated bladder cancer risks. Further exploration of dietary SFN in the prevention and/or treatment of bladder cancer may help facilitate a paradigm shift in clinicians' use of food as medicine and encourage enhanced research and prescription of nutrition-based oncology interventions [181]. Currently, a prospective study designed specifically to evaluate the impact of cruciferous vegetable intake on bladder cancer prognosis in the context of polymorphic ITC-metabolizing genes is underway [182]. Clinical trials to examine the effect of SFN or SFN-rich dietary supplements alone or in combination with other therapies in preventing bladder cancer recurrence are also warranted.

Future studies of SFN in bladder cancer can emphasize supplemental cancer therapy and prevention. For cancer treatment, SFN can be used either as an alternative therapeutic agent or as a supplementary agent in combination with other chemo- or immunotherapies. SFN in food extract format, e.g., broccoli sprout extract, is commercially available, facilitating the clinical investigation of SFN in the patient population [183]. While cancer treatment with SFN is possible, the wider implication of SFN as a therapeutic agent may be its potential to prevent primary or secondary cancers among high-risk populations.

Cruciferous vegetables are a rich dietary source of SFN and are widely available in the US. As the initiatives behind food as medicine expand, cruciferous vegetables could serve as a low-cost and low-toxicity regimen for long-term use among populations at high risk of developing cancer due to a history of heavy exposure to smoking and carcinogens. Given the high recurrence risk that early-stage bladder cancer survivors face, studies on how to engage and encourage patients to increase their consumption of cruciferous vegetables to improve cancer outcomes are ongoing [184]. Overall, these lines of research may offer evidence-based dietary recommendations to fight against carcinogen-induced cancers and can also have wider-reaching and paradigm-shifting effects on how modern medicine uses nutrition to tackle prevention and treatment challenges.

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Disclosure of conflict of interest

None.

Abbreviations

BBN, N-butyl-N-(4-hydroxybutyl) nitrosamine; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; SFN, Sulforaphane; TURBT, transurethral resection of bladder tumor.

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References

[1] Cancer stat facts: bladder cancer. seer.cancer.gov: NIH - National Cancer Institute.

[2] Key statistics for bladder cancer. cancer.org: American Cancer Society; 2021.

[3] Dietrich B and Srinivas S. Urothelial carcinoma: the evolving landscape of immunotherapy for patients with advanced disease. Res Rep Urol 2018; 10: 7-16.

[4] Apolo A and Dahut W. The Bethesda Handbook of Clinical Oncology. Philadelphia, PA 19106: Lippincott Williams & Wilkins; 2014.

[5] Park JC, Citrin DE, Agarwal PK and Apolo AB. Multimodal management of muscle-invasive bladder cancer. Curr Probl Cancer 2014; 38: 80-108.

[6] Bladder cancer: statistics. cancer.net: ASCO; 2022.

[7] Survival rates for bladder cancer. cancer.org: The American Cancer Society; 2021.

[8] Bladder cancer risk factors. cancer.org: The American Cancer Society; 2019.

[9] Janković S and Radosavljević V. Risk factors for bladder cancer. Tumori 2007; 93: 4-12.

[10] Michaud DS, Spiegelman D, Clinton SK, Rimm EB, Willett WC and Giovannucci EL. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J Natl Cancer Inst 1999; 91: 605-613.

[11] Curigliano G, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A and Santella RM. Immunohistochemical quantitation of 4-aminobiphenyl-DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. Carcinogenesis 1996; 17: 911-916.

[12] Kogevinas M, t'Mannetje A, Cordier S, Ranft U, González CA, Vineis P, Chang-Claude J, Lynge E, Wahrendorf J, Tzonou A, Jöckel KH, Serra C, Porru S, Hours M, Greiser E and Boffetta P. Occupation and bladder cancer among men in Western Europe. Cancer Causes Control 2003; 14: 907-914.

[13] Griffiths TR; Action on Bladder Cancer. Current perspectives in bladder cancer management. Int J Clin Pract 2013; 67: 435-48.

[14] Aziz MM, Raza MA, Saleem H, Wajid M, Bashir K and Ur Rehman MI. Medicinal values of herbs and plants, importance of phytochemical evaluation and ethnopharmacological screening: an illustrated review essay. Journal of pharmaceutical and cosmetic sciences. researchgate.net: Topclass Global Journals; 2014. pp. 6-10.

[15] War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S and Sharma HC. Mechanisms of plant defense against insect herbivores. Plant Signal Behav 2012; 7: 1306-1320.

[16] Jones JD and Dangl JL. The plant immune system. Nature 2006; 444: 323-329.

[17] Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry 2001; 56: 237-243.

[18] Choudhari AS, Mandave PC, Deshpande M, Ranjekar P and Prakash O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. Front Pharmacol 2020; 10: 1614.

[19] Taraphdar AK, Roy M and Bhattacharya RK. Natural products as inducers of apoptosis: implication for cancer therapy and prevention. Curr Sci 2001; 80: 1387-1396.

[20] Sun J, Chu YF, Wu X and Liu RH. Antioxidant and antiproliferative activities of common

fruits. J Agric Food Chem 2002; 50: 7449-7454.
[21] Saklani A and Kutty SK. Plant-derived compounds in clinical trials. Drug Discov Today 2008; 13: 161-171.
[22] Lam TK, Gallicchio L, Lindsley K, Shiels M, Hammond E, Tao XG, Chen L, Robinson KA, Caulfield LE, Herman JG, Guallar E and Alberg AJ. Cruciferous vegetable consumption and lung cancer risk: a systematic review. Cancer Epidemiol Biomarkers Prev 2009; 18: 184-195.
[23] Wu X, Zhou QH and Xu K. Are isothiocyanates potential anti-cancer drugs? Acta Pharmacol Sin 2009; 30: 501-512.
[24] Dixon GR. Vegetable brassicas and related crucifers. CAB International; 2007.
[25] Singh G, Kawatra A and Sehgal S. Nutritional composition of selected green leafy vegetables, herbs and carrots. Plant Foods Hum Nutr 2001; 56: 359-364.
[26] Bones AM and Rossiter JT. The myrosinase-glucosinolate system, its organisation and biochemistry. Physiologia Plantarum; 1996. pp. 194-208.
[27] Bones AM and Rossiter JT. The enzymic and chemically induced decomposition of glucosinolates. Phytochemistry 2006; 67: 1053-1067.
[28] Shapiro TA, Fahey JW, Wade KL, Stephenson KK and Talalay P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. Cancer Epidemiol Biomarkers Prev 1998; 7: 1091-1100.
[29] Keum YS, Jeong WS and Kong AN. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutat Res 2004; 555: 191-202.
[30] Fahey JW, Holtzclaw WD, Wehage SL, Wade KL, Stephenson KK and Talalay P. Sulforaphane bioavailability from glucoraphanin-rich broccoli: control by active endogenous myrosinase. PLoS One 2015; 10: e0140963.
[31] Fahey JW, Wade KL, Stephenson KK, Panjwani AA, Liu H, Cornblatt G, Cornblatt BS, Ownby SL, Fuchs E, Holtzclaw WD and Cheskin LJ. Bioavailability of sulforaphane following ingestion of glucoraphanin-rich broccoli sprout and seed extracts with active myrosinase: a pilot study of the effects of proton pump inhibitor administration. Nutrients 2019; 11: 1489.
[32] Palliyaguru DL, Yuan JM, Kensler TW and Fahey JW. Isothiocyanates: translating the power of plants to people. Mol Nutr Food Res 2018; 62: e1700965.
[33] Pernice R, Hauder J, Koehler P, Vitaglione P, Fogliano V and Somoza V. Effect of sulforaphane on glutathione-adduct formation and on glutathione_S_transferase-dependent detoxification of acrylamide in Caco-2 cells. Mol Nutr Food Res 2009; 53: 1540-1550.
[34] Townsend DM and Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003; 22: 7369-7375.
[35] Gasper AV, Al-Janobi A, Smith JA, Bacon JR, Fortun P, Atherton C, Taylor MA, Hawkey CJ, Barrett DA and Mithen RF. Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. Am J Clin Nutr 2005; 82: 1283-1291.
[36] Hayes JD and Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2000; 61: 154-166.
[37] Becker TM and Juvik JA. The role of glucosinolate hydrolysis products from brassica vegetable consumption in inducing antioxidant activity and reducing cancer incidence. Diseases 2016; 4: 22.
[38] Piacentini S, Polimanti R, Porreca F, Martínez-Labarga C, De Stefano GF and Fuciarelli M. GSTT1 and GSTM1 gene polymorphisms in European and African populations. Mol Biol Rep 2011; 38: 1225-1230.
[39] Lampe JW. Interindividual differences in response to plant-based diets: implications for cancer risk. Am J Clin Nutr 2009; 89: 1553S-1557S.
[40] Wang LI, Giovannucci EL, Hunter D, Neuberg D, Su L and Christiani DC. Dietary intake of Cruciferous vegetables, Glutathione S-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. Cancer Causes Control 2004; 15: 977-85.
[41] Gigliotti JC, Tin A, Pourafshar S, Cechova S, Wang YT, Sung SJ, Bodonyi-Kovacs G, Cross JV, Yang G, Nguyen N, Chan F, Rebholz C, Yu B, Grove ML, Grams ME, Köttgen A, Scharpf R, Ruiz P, Boerwinkle E, Coresh J and Le TH. GSTM1 deletion exaggerates kidney injury in experimental mouse models and confers the protective effect of cruciferous vegetables in mice and humans. J Am Soc Nephrol 2020; 31: 102-116.
[42] London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK and Yu MC. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet 2000; 356: 724-9.
[43] Steck SE, Gaudet MM, Britton JA, Teitelbaum SL, Terry MB, Neugut AI, Santella RM and Gammon MD. Interactions among GSTM1, GSTT1 and GSTP1 polymorphisms, cruciferous vegetable intake and breast cancer risk. Carcinogenesis 2007; 28: 1954-1959.

[44] Ambrosone CB and Tang L. Cruciferous vegetable intake and cancer prevention: role of nutrigenetics. Cancer Prev Res (Phila) 2009; 2: 298-300.

[45] 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 and Mori M. A phase II study of sulforaphane-rich broccoli sprout extracts in men with recurrent prostate cancer. Invest New Drugs 2015; 33: 480-489.

[46] Ritz SA, Wan J and Diaz-Sanchez D. Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. Am J Physiol Lung Cell Mol Physiol 2007; 292: L33-L39.

[47] Bhattacharjee P, Paul S, Banerjee M, Patra D, Banerjee P, Ghoshal N, Bandyopadhyay A and Giri AK. Functional compensation of glutathione S-transferase M1 (GSTM1) null by another GST superfamily member, GSTM2. Sci Rep 2013; 3: 2704.

[48] Wang Y, Wu H, Dong N, Su X, Duan M, Wei Y, Wei J, Liu G, Peng Q and Zhao Y. Sulforaphane induces S-phase arrest and apoptosis via p53-dependent manner in gastric cancer cells. Sci Rep 2021; 11: 2504.

[49] Kensler TW, Egner PA, Agyeman AS, Visvanathan K, Groopman JD, Chen JG, Chen TY, Fahey JW and Talalay P. Keap1-nrf2 signaling: a target for cancer prevention by sulforaphane. Top Curr Chem 2013; 329: 163-77.

[50] Wang X, Li Y, Dai Y, Liu Q, Ning S, Liu J, Shen Z, Zhu D, Jiang F, Zhang J and Li Z. Sulforaphane improves chemotherapy efficacy by targeting cancer stem cell-like properties via the miR-124/IL-6R/STAT3 axis. Sci Rep 2016; 6: 36796.

[51] Jabbarzadeh Kaboli P, Afzalipour Khoshkbejari M, Mohammadi M, Abiri A, Mokhtarian R, Vazifemand R, Amanollahi S, Yazdi Sani S, Li M, Zhao Y, Wu X, Shen J, Cho CH and Xiao Z. Targets and mechanisms of sulforaphane derivatives obtained from cruciferous plants with special focus on breast cancer - contradictory effects and future perspectives. Biomed Pharmacother 2020; 121: 109635.

[52] Nioi P, McMahon M, Itoh K, Yamamoto M and Hayes JD. Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. Biochem J 2003; 374: 337-348.

[53] Yamamoto M, Kensler TW and Motohashi H. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 2018; 98: 1169-1203.

[54] Dinkova-Kostova AT, Fahey JW, Kostov RV and Kensler TW. KEAP1 and done? Targeting the NRF2 pathway with sulforaphane. Trends Food Sci Technol 2017; 69: 257-69.

[55] Li D, Shao R, Wang N, Zhou N, Du K, Shi J, Wang Y, Zhao Z, Ye X, Zhang X and Xu H. Sulforaphane activates a lysosome-dependent transcriptional program to mitigate oxidative stress. Autophagy 2021; 17: 872-887.

[56] Moi P, Chan K, Asunis I, Cao A and Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci U S A 1994; 91: 9926-9930.

[57] Venugopal R and Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A 1996; 93: 14960-14965.

[58] Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M and Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997; 236: 313-322.

[59] Sekhar KR, Yan XX and Freeman ML. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. Oncogene 2002; 21: 6829-6834.

[60] Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, Uchida K and Yamamoto M. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol Cell Biol 2006; 26: 221-229.

[61] Ma Q. Xenobiotic-activated receptors: from transcription to drug metabolism to disease. Chem Res Toxicol 2008; 21: 1651-1671.

[62] Kobayashi A, Ohta T and Yamamoto M. Unique function of the Nrf2-Keap1 pathway in the inducible expression of antioxidant and detoxifying enzymes. Methods Enzymol 2004; 378: 273-286.

[63] Nebert DW and Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer 2006; 6: 947-960.

[64] Reed L, Arlt VM and Phillips DH. The role of cytochrome P450 enzymes in carcinogen activation and detoxication: an in vivo-in vitro paradox. Carcinogenesis 2018; 39: 851-859.

[65] Jancova P, Anzenbacher P and Anzenbacherova E. Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2010; 154: 103-116.

[66] Mahéo K, Morel F, Langouët S, Kramer H, Le Ferrec E, Ketterer B and Guillouzo A. Inhibition of cytochromes P-450 and induction of glutathione S-transferases by sulforaphane in primary human and rat hepatocytes. Cancer Res 1997; 57: 3649-3652.
[67] Barcelo S, Gardiner JM, Gescher A and Chipman JK. CYP2E1-mediated mechanism of antigenotoxicity of the broccoli constituent sulforaphane. Carcinogenesis 1996; 17: 277-282.
[68] Almazroo OA, Miah MK and Venkataramanan R. Drug metabolism in the liver. Clin Liver Dis 2017; 21: 1-20.
[69] Liu P, Wang W, Tang J, Bowater RP and Bao Y. Antioxidant effects of sulforaphane in human HepG2 cells and immortalised hepatocytes. Food Chem Toxicol 2019; 128: 129-136.
[70] Liu M, Yao XD, Li W, Geng J, Yan Y, Che JP, Xu YF and Zheng JH. Nrf2 sensitizes prostate cancer cells to radiation via decreasing basal ROS levels. Biofactors 2015; 41: 52-57.
[71] Coussens LM and Werb Z. Inflammation and cancer. Nature 2002; 420: 860-867.
[72] Kuper H, Adami HO and Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med 2000; 248: 171-183.
[73] Stefanson AL and Bakovic M. Dietary regulation of Keap1/Nrf2/ARE pathway: focus on plant-derived compounds and trace minerals. Nutrients 2014; 6: 3777-3801.
[74] Atwell LL, Zhang Z, Mori M, Farris P, Vetto JT, Naik AM, Oh KY, Thuillier P, Ho E and Shannon J. Sulforaphane bioavailability and chemopreventive activity in women scheduled for breast biopsy. Cancer Prev Res (Phila) 2015; 8: 1184-1191.
[75] Liang J, Jahraus B, Balta E, Ziegler JD, Hübner K, Blank N, Niesler B, Wabnitz GH and Samstag Y. Sulforaphane inhibits inflammatory responses of primary human T-cells by increasing ROS and depleting glutathione. Front Immunol 2018; 9: 2584.
[76] Wang Y, Mandal AK, Son YO, Pratheeshkumar P, Wise JTF, Wang L, Zhang Z, Shi X and Chen Z. Roles of ROS, Nrf2, and autophagy in cadmium-carcinogenesis and its prevention by sulforaphane. Toxicol Appl Pharmacol 2018; 353: 23-30.
[77] Han Y, Runge MS and Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. Circ Res 1999; 84: 695-703.
[78] Shi G, Li D, Fu J, Sun Y, Li Y, Qu R, Jin X and Li D. Upregulation of cyclooxygenase-2 is associated with activation of the alternative nuclear factor kappa B signaling pathway in colonic adenocarcinoma. Am J Transl Res 2015; 7: 1612-1620.
[79] Shan Y, Lin N, Yang X, Tan J, Zhao R, Dong S and Wang S. Sulphoraphane inhibited the expressions of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 through MyD88-dependent toll-like receptor-4 pathway in cultured endothelial cells. Nutr Metab Cardiovasc Dis 2012; 22: 215-222.
[80] Woods CG, Fu J, Xue P, Hou Y, Pluta LJ, Yang L, Zhang Q, Thomas RS, Andersen ME and Pi J. Dose-dependent transitions in Nrf2-mediated adaptive response and related stress responses to hypochlorous acid in mouse macrophages. Toxicol Appl Pharmacol 2009; 238: 27-36.
[81] Qin Z, Tang J, Han P, Jiang X, Yang C, Li R, Tang M, Shen B, Wang W, Qin C and Zhang W. Protective effects of sulforaphane on di-n-butylphthalate-induced testicular oxidative stress injury in male mice offsprings via activating Nrf2/ARE pathway. Oncotarget 2017; 8: 82956-82967.
[82] Heiss E, Herhaus C, Klimo K, Bartsch H and Gerhäuser C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. J Biol Chem 2001; 276: 32008-32015.
[83] Shams R, Abu-Khudir R and Ali EM. Sulforaphane, polyphenols and related anti-inflammatory and antioxidant activities changes of Egyptian broccoli during growth. Food Measure 2017; 11: 2061-2068.
[84] Woo KJ and Kwon TK. Sulforaphane suppresses lipopolysaccharide-induced cyclooxygenase-2 (COX-2) expression through the modulation of multiple targets in COX-2 gene promoter. Int Immunopharmacol 2007; 7: 1776-1783.
[85] Subedi L, Lee JH, Yumnam S, Ji E and Kim SY. Anti-inflammatory effect of sulforaphane on LPS-activated microglia potentially through JNK/AP-1/NF-κB inhibition and Nrf2/HO-1 activation. Cells 2019; 8: 194.
[86] Hahm ER and Singh SV. Sulforaphane inhibits constitutive and interleukin-6-induced activation of signal transducer and activator of transcription 3 in prostate cancer cells. Cancer Prev Res (Phila) 2010; 3: 484-494.
[87] Thejass P and Kuttan G. Augmentation of natural killer cell and antibody-dependent cellular cytotoxicity in BALB/c mice by sulforaphane, a naturally occurring isothiocyanate from broccoli through enhanced production of cytokines IL-2 and IFN-gamma. Immunopharmacol Immunotoxicol 2006; 28: 443-457.
[88] Pal S and Konkimalla VB. Sulforaphane regulates phenotypic and functional switching of both induced and spontaneously differentiating human monocytes. Int Immunopharmacol 2016; 35: 85-98.

[89] Ali M, Bonay M, Vanhee V, Vinit S and Deramaudt TB. Comparative effectiveness of 4 natural and chemical activators of Nrf2 on inflammation, oxidative stress, macrophage polarization, and bactericidal activity in an in vitro macrophage infection model. PLoS One 2020; 15: e0234484.
[90] Sun Y, Tang J, Li C, Liu J and Liu H. Sulforaphane attenuates dextran sodium sulphate induced intestinal inflammation via IL-10/STAT3 signaling mediated macrophage phenotype switching. Food Science and Human Wellness 2022; 11: 129-142.
[91] Martinez FO and Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014; 6: 13.
[92] Mills CD. M1 and M2 macrophages: oracles of health and disease. Crit Rev Immunol 2012; 32: 463-488.
[93] Shen C, Zhang Z, Tian Y, Li F, Zhou L, Jiang W, Yang L, Zhang B, Wang L and Zhang Y. Sulforaphane enhances the antitumor response of chimeric antigen receptor T cells by regulating PD-1/PD-L1 pathway. BMC Med 2021; 19: 283.
[94] Myzak MC, Tong P, Dashwood WM, Dashwood RH and Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. Exp Biol Med (Maywood) 2007; 232: 227-234.
[95] Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MÁ, Ahn N, Imhof A, Caldas C, Jenuwein T and Esteller M. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005; 37: 391-400.
[96] Barneda-Zahonero B and Parra M. Histone deacetylases and cancer. Mol Oncol 2012; 6: 579-89.
[97] Robert T, Vanoli F, Chiolo I, Shubassi G, Bernstein KA, Rothstein R, Botrugno OA, Parazzoli D, Oldani A, Minucci S and Foiani M. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature 2011; 471: 74-79.
[98] Gerhauser C. Epigenetic impact of dietary isothiocyanates in cancer chemoprevention. Curr Opin Clin Nutr Metab Care 2013; 16: 405-410.
[99] Tortorella SM, Royce SG, Licciardi PV and Karagiannis TC. Dietary sulforaphane in cancer chemoprevention: the role of epigenetic regulation and HDAC inhibition. Antioxid Redox Signal 2015; 22: 1382-1424.
[100] Kaufman-Szymczyk A, Majewski G, Lubecka-Pietruszewska K and Fabianowska-Majewska K. The role of sulforaphane in epigenetic mechanisms, including interdependence between histone modification and DNA methylation. Int J Mol Sci 2015; 16: 29732-29743.
[101] Jiang LL, Zhou SJ, Zhang XM, Chen HQ and Liu W. Sulforaphane suppresses in vitro and in vivo lung tumorigenesis through downregulation of HDAC activity. Biomed Pharmacother 2016; 78: 74-80.
[102] Clarke JD, Hsu A, Yu Z, Dashwood RH and Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. Mol Nutr Food Res 2011; 55: 999-1009.
[103] Cheng YM, Tsai CC and Hsu YC. Sulforaphane, a dietary isothiocyanate, induces G2/M arrest in cervical cancer cells through CyclinB1 downregulation and GADD45β/CDC2 association. Int J Mol Sci 2016; 17: 1530.
[104] Żuryń A, Litwiniec A, Safiejko-Mroczka B, Klimaszewska-Wiśniewska A, Gagat M, Krajewski A, Gackowska L and Grzanka D. The effect of sulforaphane on the cell cycle, apoptosis and expression of cyclin D1 and p21 in the A549 non-small cell lung cancer cell line. Int J Oncol 2016; 48: 2521-2533.
[105] Santos PW, Machado ART, De Grandis R, Ribeiro DL, Tuttis K, Morselli M, Aissa AF, Pellegrini M and Antunes LMG. Effects of sulforaphane on the oxidative response, apoptosis, and the transcriptional profile of human stomach mucosa cells in vitro. Mutat Res Genet Toxicol Environ Mutagen 2020; 854-855: 503201.
[106] Cheung KL and Kong AN. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. AAPS J 2010; 12: 87-97.
[107] Singh SV, Srivastava SK, Choi S, Lew KL, Antosiewicz J, Xiao D, Zeng Y, Watkins SC, Johnson CS, Trump DL, Lee YJ, Xiao H and Herman-Antosiewicz A. Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. J Biol Chem 2005; 280: 19911-19924.
[108] Park SY, Kim GY, Bae SJ, Yoo YH and Choi YH. Induction of apoptosis by isothiocyanate sulforaphane in human cervical carcinoma HeLa and hepatocarcinoma HepG2 cells through activation of caspase-3. Oncol Rep 2007; 18: 181-187.
[109] Joseph P and Jaiswal AK. NAD(P)H:quinone oxidoreductase 1 (DT diaphorase) specifically prevents the formation of bezo[a]pyrene quinone-DNA adducts generated by cytochrome P4501A1 and P450 reductase. Proc Natl Acad Sci U S A 1994; 91: 8413-7.
[110] Singletary K and MacDonald C. Inhibition of benzo[a]pyrene- and 1,6-dinitropyrene-DNA

adduct formation in human mammary epithelial cells bydibenzoylmethane and sulforaphane. Cancer Lett 2000; 155: 47-54.
[111] Ferreira de Oliveira JM, Remédios C, Oliveira H, Pinto P, Pinho F, Pinho S, Costa M and Santos C. Sulforaphane induces DNA damage and mitotic abnormalities in human osteosarcoma MG-63 cells: correlation with cell cycle arrest and apoptosis. Nutr Cancer 2014; 66: 325-334.
[112] Zanichelli F, Capasso S, Di Bernardo G, Cipollaro M, Pagnotta E, Cartenì M, Casale F, Iori R, Giordano A and Galderisi U. Low concentrations of isothiocyanates protect mesenchymal stem cells from oxidative injuries, while high concentrations exacerbate DNA damage. Apoptosis 2012; 17: 964-974.
[113] Yu D, Sekine-Suzuki E, Xue L, Fujimori A, Kubota N and Okayasu R. Chemopreventive agent sulforaphane enhances radiosensitivity in human tumor cells. Int J Cancer 2009; 125: 1205-1211.
[114] Bacon JR, Williamson G, Garner RC, Lappin G, Langouët S and Bao Y. Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. Carcinogenesis 2003; 24: 1903-1911.
[115] Urinary Bladder. Urinary System.training.seer.cancer.gov: National Institutes of Health.
[116] Young B, Woodford P and O'Dowd G. Wheater's functional histology E-book: a text and colour atlas. Elsevier Health Sciences 2013.
[117] Layers of the Bladder Wall. SEER training modules.training.seer.cancer.gov: NIH National Cancer Institute.
[118] Khandelwal P, Abraham SN and Apodaca G. Cell biology and physiology of the uroepithelium. Am J Physiol Renal Physiol 2009; 297: F1477-1501.
[119] Carattino MD, Prakasam HS, Ruiz WG, Clayton DR, McGuire M, Gallo LI and Apodaca G. Bladder filling and voiding affect umbrella cell tight junction organization and function. Am J Physiol Renal Physiol 2013; 305: F1158-F1168.
[120] Bolla S, Odeluga N and Jetti R. Histology, bladder. StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
[121] What is bladder cancer? Bladder cancer. cancer.org: The American Cancer Society; 2019.
[122] Curtius K, Wright NA and Graham TA. An evolutionary perspective on field cancerization. Nat Rev Cancer 2018; 18: 19-32.
[123] Dakubo GD, Jakupciak JP, Birch-Machin MA and Parr RL. Clinical implications and utility of field cancerization. Cancer Cell Int 2007; 7: 2.
[124] Srivastava S, Ghosh S, Kagan J and Mazurchuk R. The PreCancer Atlas (PCA). Trends Cancer 2018; 4: 513-514.
[125] Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, Samuel M, Ritchie EK, Guzman ML, Ballman KV, Roboz GJ and Hassane DC. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med 2018; 24: 1015-1023.
[126] Strandgaard T, Nordentoft I, Lamy P, Christensen E, Thomsen MBH, Jensen JB and Dyrskjot L. Mutational analysis of field cancerization in bladder cancer. Bladder Cancer 2020; 253-64.
[127] Jones TD, Wang M, Eble JN, MacLennan GT, Lopez-Beltran A, Zhang S, Cocco A and Cheng L. Molecular evidence supporting field effect in urothelial carcinogenesis. Clin Cancer Res 2005; 11: 6512-6519.
[128] Babayan A, Andreeva Y, Zaletaev D and Nemtsova M. Molecular evidence supporting field effect in recurrent and primary multiple superficial bladder cancers. Cancer Genet Cytogenet 2010; 203: 82.
[129] Mori K, Mostafaei H, Abufaraj M, Yang L, Egawa S and Shariat SF. Smoking and bladder cancer: review of the recent literature. Curr Opin Urol 2020; 30: 720-725.
[130] Silverman DT, Hartge P, Morrison AS and Devesa SS. Epidemiology of bladder cancer. Hematol Oncol Clin North Am 1992; 6: 1-30.
[131] Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A and Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA 2011; 306: 737-745.
[132] Joshi M, Millis SZ, Arguello D, Holder SL, Lamm D, Reddy S, Belani C, Drabick JJ and Vogelzang NJ. Molecular characterization of bladder cancer in smokers versus nonsmokers. Eur Urol Focus 2018; 4: 94-97.
[133] Kelsey KT, Hirao T, Schned A, Hirao S, Devi-Ashok T, Nelson HH, Andrew A and Karagas MR. A population-based study of immunohistochemical detection of p53 alteration in bladder cancer. Br J Cancer 2004; 90: 1572-1576.
[134] Habuchi T, Devlin J, Elder PA and Knowles MA. Detailed deletion mapping of chromosome 9q in bladder cancer: evidence for two tumour suppressor loci. Oncogene 1995; 11: 1671-1674.
[135] van Tilborg AA, Groenfeld LE, van der Kwast TH and Zwarthoff EC. Evidence for two candidate tumour suppressor loci on chromosome 9q in transitional cell carcinoma (TCC) of the bladder but no homozygous deletions in bladder tumour cell lines. Br J Cancer 1999; 80: 489-494.
[136] Hirao S, Hirao T, Marsit CJ, Hirao Y, Schned A, Devi-AShok T, Nelson HH, Andrew A, Karagas MR and Kelsey KT. Loss of heterozygosity on

chromosome 9q and p53 alterations in human bladder cancer. Cancer 2005; 104: 1918-23.
[137] Tripathi N, Sayegh N, Jo Y, Li H, Nussenzveig R, Haaland B, Thomas V, Gupta S, Sirohi D, Swami U, Agarwal N and Maughan B. Tumor genomic landscape in smokers compared to non-smoker patients with locally advanced or metastatic urothelial carcinoma. J Clin Oncol 2022; 40: 554.
[138] Volanis D, Kadiyska T, Galanis A, Delakas D, Logotheti S and Zoumpourlis V. Environmental factors and genetic susceptibility promote urinary bladder cancer. Toxicol Lett 2010; 193: 131-137.
[139] Letašiová S, Medve'ová A, Šovčíková A, Dušinská M, Volkovová K, Mosoiu C and Bartonová A. Bladder cancer, a review of the environmental risk factors. Environ Health 2012; 11 Suppl 1: S11.
[140] Rouissi K, Ouerhani S, Marrakchi R, Ben Slama MR, Sfaxi M, Ayed M, Chebil M and El Gaaied AB. Combined effect of smoking and inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1 on bladder cancer in a Tunisian population. Cancer Genet Cytogenet 2009; 190: 101-107.
[141] Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, Ye L and Talalay P. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. Nutr Cancer 2006; 55: 53-62.
[142] Su X, Jiang X, Meng L, Dong X, Shen Y and Xin Y. Anticancer activity of sulforaphane: the epigenetic mechanisms and the Nrf2 signaling pathway. Oxid Med Cell Longev 2018; 2018: 5438179.
[143] Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: a report of the surgeon general. Atlanta (GA): Centers for Disease Control and Prevention (US); 2010.
[144] Lung cancer: what are the risk factors. cdc.gov: Centers for Disease Control and Prevention; 2020.
[145] Jalas JR, Hecht SS and Murphy SE. Cytochrome P450 enzymes as catalysts of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. Chem Res Toxicol 2005; 18: 95-110.
[146] Vlahos R, Bozinovski S, Jones JE, Powell J, Gras J, Lilja A, Hansen MJ, Gualano RC, Irving L and Anderson GP. Differential protease, innate immunity, and NF-kappaB induction profiles during lung inflammation induced by subchronic cigarette smoke exposure in mice. Am J Physiol Lung Cell Mol Physiol 2006; 290: L931-945.
[147] Meylan E, Dooley AL, Feldser DM, Shen L, Turk E, Ouyang C and Jacks T. Requirement for NF-kappaB signalling in a mouse model of lung adenocarcinoma. Nature 2009; 462: 104-107.
[148] Talalay P and Fahey JW. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. J Nutr 2001; 131: 3027S-3033S.
[149] Zhao H, Lin J, Grossman HB, Hernandez LM, Dinney CP and Wu X. Dietary isothiocyanates, GSTM1, GSTT1, NAT2 polymorphisms and bladder cancer risk. Int J Cancer 2007; 120: 2208-2213.
[150] Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB and McCann SE. Intake of cruciferous vegetables modifies bladder cancer survival. Cancer Epidemiol Biomarkers Prev 2010; 19: 1806-11.
[151] Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB and McCann SE. Consumption of raw cruciferous vegetables is inversely associated with bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2008; 17: 938-944.
[152] Wang Z, Kwan ML, Pratt R, Roh JM, Kushi LH, Danforth KN, Zhang Y, Ambrosone CB and Tang L. Effects of cooking methods on total isothiocyanate yield from cruciferous vegetables. Food Sci Nutr 2020; 8: 5673-82.
[153] Zhang Y. Cancer-preventive isothiocyanates: measurement of human exposure and mechanism of action. Mutat Res 2004; 555: 173-190.
[154] Munday R, Mhawech-Fauceglia P, Munday CM, Paonessa JD, Tang L, Munday JS, Lister C, Wilson P, Fahey JW, Davis W and Zhang Y. Inhibition of urinary bladder carcinogenesis by broccoli sprouts. Cancer Res 2008; 68: 1593-600.
[155] Ye L, Dinkova-Kostova AT, Wade KL, Zhang Y, Shapiro TA and Talalay P. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. Clin Chim Acta 2002; 316: 43-53.
[156] Iida K, Itoh K, Maher JM, Kumagai Y, Oyasu R, Mori Y, Shimazui T, Akaza H and Yamamoto M. Nrf2 and p53 cooperatively protect against BBN-induced urinary bladder carcinogenesis. Carcinogenesis 2007; 28: 2398-2403.
[157] Iida K, Itoh K, Kumagai Y, Oyasu R, Hattori K, Kawai K, Shimazui T, Akaza H and Yamamoto M. Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. Cancer Res 2004; 64: 6424-6431.
[158] Ding Y, Paonessa JD, Randall KL, Argoti D, Chen L, Vouros P and Zhang Y. Sulforaphane

inhibits 4-aminobiphenyl-induced DNA damage in bladder cells and tissues. Carcinogenesis 2010; 31: 1999-2003.
[159] Zhang DD and Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 2003; 23: 8137-8151.
[160] Jo GH, Kim GY, Kim WJ, Park KY and Choi YH. 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 2014; 45: 1497-1506.
[161] Tang L, Zhang Y, Jobson HE, Li J, Stephenson KK, Wade KL and Fahey JW. Potent activation of mitochondria-mediated apoptosis and arrest in S and M phases of cancer cells by a broccoli sprout extract. Mol Cancer Ther 2006; 5: 935-944.
[162] Tang L and Zhang Y. Dietary isothiocyanates inhibit the growth of human bladder carcinoma cells. J Nutr 2004; 134: 2004-2010.
[163] Wang F and 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.
[164] Kerns ML, Miller RJ, Mazhar M, Byrd AS, Archer NK, Pinkser BL, Lew L, Dillen CA, Wang R, Miller LS, Chien AL and Kang S. Pathogenic and therapeutic role for NRF2 signaling in ultraviolet light-induced skin pigmentation. JCI Insight 2020; 5: e139342.
[165] Milowsky MI, Iyer G, Regazzi AM, Al-Ahmadie H, Gerst SR, Ostrovnaya I, Gellert LL, Kaplan R, Garcia-Grossman IR, Pendse D, Balar AV, Flaherty AM, Trout A, Solit DB and Bajorin DF. Phase II study of everolimus in metastatic urothelial cancer. BJU Int 2013; 112: 462-470.
[166] Justin S, Rutz J, Maxeiner S, Chun FK, Juengel E and Blaheta RA. Bladder cancer metastasis induced by chronic everolimus application can be counteracted by sulforaphane in vitro. Int J Mol Sci 2020; 21: 5582.
[167] Kerr C, Adhikary G, Grun D, George N and Eckert RL. Combination cisplatin and sulforaphane treatment reduces proliferation, invasion, and tumor formation in epidermal squamous cell carcinoma. Mol Carcinog 2018; 57: 3-11.
[168] Calcabrini C, Maffei F, Turrini E and Fimognari C. Sulforaphane potentiates anticancer effects of doxorubicin and cisplatin and mitigates their toxic effects. Front Pharmacol 2020; 11: 567.
[169] Dinkova-Kostova AT, Jenkins SN, Fahey JW, Ye L, Wehage SL, Liby KT, Stephenson KK, Wade KL and Talalay P. Protection against UV-light-induced skin carcinogenesis in SKH-1 high-risk mice by sulforaphane-containing broccoli sprout extracts. Cancer Lett 2006; 240: 243-252.
[170] Balasubramanian S, Chew YC and Eckert RL. Sulforaphane suppresses polycomb group protein level via a proteasome-dependent mechanism in skin cancer cells. Mol Pharmacol 2011; 80: 870-878.
[171] Ma Q. Xenobiotic-activated receptors: from transcription to drug metabolism to disease. Chem Res Toxicol 2008; 21: 1651-1671.
[172] Reed JR, Leon RP, Hall MK and Schwertfeger KL. Interleukin-1beta and fibroblast growth factor receptor 1 cooperate to induce cyclooxygenase-2 during early mammary tumourigenesis. Breast Cancer Res 2009; 11: R21.
[173] Sharma C, Sadrieh L, Priyani A, Ahmed M, Hassan AH and Hussain A. Anti-carcinogenic effects of sulforaphane in association with its apoptosis-inducing and anti-inflammatory properties in human cervical cancer cells. Cancer Epidemiol 2011; 35: 272-278.
[174] Kerns ML, Miller RJ, Mazhar M, Byrd AS, Archer NK, Pinkser BL, Lew L, Dillen CA, Wang R, Miller LS, Chien AL and Kang S. Pathogenic and therapeutic role for NRF2 signaling in ultraviolet light-induced skin pigmentation. JCI Insight 2020; 5: e139342.
[175] Alyoussef A and Taha M. Antitumor activity of sulforaphane in mice model of skin cancer via blocking sulfatase-2. Exp Dermatol 2019; 28: 28-34.
[176] Broccoli and cruciferous vegetables: reduce overall cancer risk. aicr.org: American Institute for Cancer Research; 2021.
[177] Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB and McCann SE. Consumption of raw cruciferous vegetables is inversely associated with bladder cancer risk. Cancer Epidemiol Biomarkers Prev 2008; 17: 938-944.
[178] Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB and McCann SE. Intake of cruciferous vegetables modifies bladder cancer survival. Cancer Epidemiol Biomarkers Prev 2010; 19: 1806-1811.
[179] Abbaoui B, Telu KH, Lucas CR, Thomas-Ahner JM, Schwartz SJ, Clinton SK, Freitas MA and Mortazavi A. The impact of cruciferous vegetable isothiocyanates on histone acetylation and histone phosphorylation in bladder cancer. J Proteomics 2017; 156: 94-103.
[180] Huang L, He C, Zheng S, Wu C, Ren M and Shan Y. AKT1/hk2 axis-mediated glucose metabolism: a novel therapeutic target of sulforaphane in bladder cancer. Mol Nutr Food Res 2022; 66: e2100738.

[181] Downer S, Berkowitz SA, Harlan TS, Olstad DL and Mozaffarian D. Food is medicine: actions to integrate food and nutrition into healthcare. BMJ 2020; 369: m2482.

[182] Kwan ML, Kushi LH, Danforth KN, Roh JM, Ergas IJ, Lee VS, Cannavale KL, Harrison TN, Contreras R, Loo RK, Aaronson DS, Quesenberry CP, Tritchler D, Ghai NR, Quinn VP, Ambrosone CB, Zhang Y and Tang L. The Be-Well Study: a prospective cohort study of lifestyle and genetic factors to reduce the risk of recurrence and progression of non-muscle-invasive bladder cancer. Cancer Causes Control 2019; 30: 187-193.

[183] Nandini DB, Rao RS, Deepak BS and Reddy PB. Sulforaphane in broccoli: the green chemoprevention!! Role in cancer prevention and therapy. J Oral Maxillofac Pathol 2020; 24: 405.

[184] Yeary KHK, Clark N, Saad-Harfouche F, Erwin D, Kuliszewski MG, Li Q, McCann SE, Yu H, Lincourt C, Zoellner J and Tang L. Cruciferous vegetable intervention to reduce the risk of cancer recurrence in non-muscle-invasive bladder cancer survivors: development using a systematic process. JMIR Cancer 2022; 8: e32291.

[185] Bacon JR, Williamson G, Garner RC, Lappin G, Langouët S and Bao Y. Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. Carcinogenesis 2003; 24: 1903-1911.

[186] Dickinson SE, Melton TF, Olson ER, Zhang J, Saboda K and Bowden GT. Inhibition of activator protein-1 by sulforaphane involves interaction with cysteine in the cFos DNA-binding domain: implications for chemoprevention of UVB-induced skin cancer. Cancer Res 2009; 69: 7103-7110.

[187] Li S, Yang Y, Sargsyan D, Wu R, Yin R, Kuo HD, Yang I, Wang L, Cheng D, Ramirez CN, Hudlikar R, Lu Y and Kong AN. Epigenome, transcriptome, and protection by sulforaphane at different stages of UVB-induced skin carcinogenesis. Cancer Prev Res (Phila) 2020; 13: 551-562.