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Mol Nutr Food Res. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Mol Nutr Food Res. 2018 September ; 62(18): e1800079. doi:10.1002/mnfr.201800079.

Cruciferous Vegetables, Isothiocyanates and Bladder Cancer Prevention

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Abstract

Bladder cancer is a significant health burden due to its high prevalence, risk of mortality, morbidity, and high cost of medical care. Epidemiologic evidence suggests that diets rich in cruciferous vegetables, particularly broccoli, are associated with lower bladder cancer risk. Phytochemicals in cruciferous vegetables, such as glucosinolates, which are enzymatically hydrolyzed to bioactive isothiocyanates, are possible mediators of an anticancer effect. *In vitro* studies have shown inhibition of bladder cancer cell lines, cell cycle arrest and induction of apoptosis by these isothiocyanates, in particular sulforaphane and erucin. Although, not yet completely understood, many mechanisms of anti-cancer activity at the steps of cancer initiation, promotion and progression have been attributed to these isothiocyanates. They target multiple pathways including the adaptive stress response, phase I/II enzyme modulation, pro-growth, -survival, -inflammatory signaling, angiogenesis, and even epigenetic modulation. Multiple *in vivo* studies have shown the bioavailability of isothiocyanates and their anti-tumoral effects. Although human studies are limited, they support oral bioavailability with reasonable plasma and urine concentrations achieved. Overall, both cell and animal studies support a potential role for isothiocyanates in bladder cancer prevention and treatment. Future studies are necessary to examine clinically relevant outcomes and define guidelines on ameliorating the bladder cancer burden.

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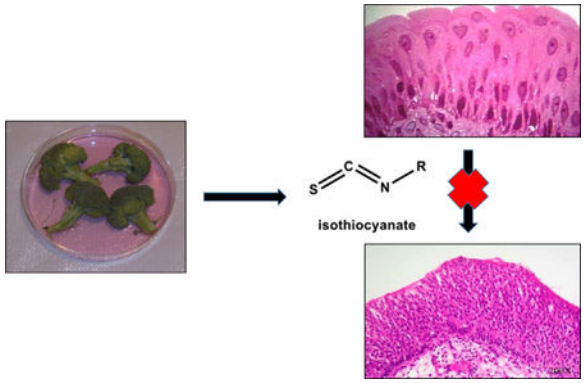
Graphical Abstract.

There is mounting evidence that isothiocyanates, derived from cruciferous vegetables, show strong promise to prevent bladder cancer, specifically transitional cell carcinoma (TCC) of the urinary bladder.



Keywords

isothiocyanates; bladder cancer; cruciferous vegetables; glucosinolates; chemoprevention

I. Introduction

Our interest in bladder cancer risk and prevention was motivated by collaboration on a series of large epidemiological studies that elucidated and quantified the potential roles of tobacco, geographic location, fluids, and dietary components on the risk of bladder cancer [1–5]. Interestingly, among the vast array of fruits and vegetables available in North America, only cruciferous vegetables emerged with significant associations with reduced bladder cancer risk. The objective of this review is to critically evaluate the accumulated literature regarding the potential role of cruciferous vegetables in bladder cancer prevention and as a possible adjunct to treatment. Cruciferous vegetables contain a complex mix of phytochemicals, however, this review will focus on isothiocyanates (ITC)s. We begin by defining the burden of bladder cancer and its pathophysiology. We then explore the cruciferous vegetable family, with a focus upon bioactive isothiocyanates, and summarize the present epidemiologic evidence for employing this class of vegetables in bladder cancer prevention and/or treatment. Next, we will define the chemistry and bioavailability of cruciferous vegetable isothiocyanates. We then proceed to review *in vitro* and *in vivo* experimental studies evaluating the effects of cruciferous vegetables and isothiocyanates in bladder cancer, followed by a synopsis of the diverse potential mechanisms of actions suggested to be at play in the bioactivity of these compounds. Finally, we summarize present findings from human interventions to assess the potential of these compounds to prevent bladder cancer and then discuss strategies for future larger scale human clinical trials and how we should proceed as a field to help ameliorate the bladder cancer burden.

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II. The Bladder Cancer Burden

Carcinoma of the urinary bladder presents a significant health care burden in the United States and around the globe. In the United States, bladder cancer is the sixth most common cancer and the eighth most common cause of cancer death, in men, with an estimated 79,000 new cases and 17,000 deaths in 2017 [6, 7]. It is the second most prevalent neoplasm in men 60 years of age or older [8–11]. Worldwide, almost 400,000 new cases are diagnosed annually with approximately 150,000 deaths [12, 13]. Strikingly, bladder cancer recurrence rate is the highest among all malignancies [14] and it has the highest lifetime treatment costs per patient, of all cancers, due to its high recurrence rate and ongoing invasive monitoring requirements [15].

Bladder cancers are derived from the urothelium, also referred to as the transitional epithelial lining of the bladder. More than 90% of bladder cancers are transitional cell carcinoma. The less common bladder cancers are squamous cell carcinoma, adenocarcinoma, and small cell carcinoma, perhaps representing different cells of origin [16, 17].

Tobacco smoking is the most important risk factor of bladder cancer, estimated to cause about half of all cases, increasing relative risk by 2- to 4-fold, and is a growing international concern due to expansion in tobacco use in developing nations [18]. Dramatically, the risk of bladder cancer is directly related to the intensity and duration of smoking, and quitting visibly reduces risk [14]. The increased risk of developing bladder cancer due to smoking is attributed to exposure to a family of known bladder carcinogens present in tobacco smoke, called aromatic amines. The mechanism of action of aromatic amines in the genesis of bladder cancer is not completely currently clear, however there has been an associated link between aromatic amine exposure and a genotoxic effect, specifically DNA adduction and mutagenicity [14]. One can also be exposed to carcinogenic aromatic amines through occupational exposure. Interestingly, bladder cancer was one of the first cancers associated with an industrial process. Epidemiological studies of urinary bladder cancers began in 1985 with a study of excessive occurrence of bladder cancer among workers in the aniline dye industry, with aniline being an aromatic amine, with then accumulating evidence clearly pointing to the relationship between bladder cancer and certain industrial chemicals with known carcinogenic effects [11]. Occupational exposure to different chemical carcinogens accounts for 10–20% of bladder cancers [19]. Some of the occupations that are linked to bladder cancer include painters, miners; and metal, rubber, leather and cement industry workers. Over 40 agents, from contemporary exposure, with a reported role in urothelial carcinogenesis have been identified, including 2-naphthylamine found in the dye and rubber industry as well as tobacco smoke and 4,4’-methylenebis[2-chloroanline] or [MBOCA] for short, which is used in polyurethane production [20]. Bladder infection with Schistosoma haematobium is a very important risk factor for bladder cancer in some parts of the world. Schistosomiasis is most prevalent in East Africa and the Middle East, and thus the most common cancer in men and the second in women [11, 21]. The chlorine [22] and arsenic in drinking water are also potential risk factors [10, 23, 24]. However, some studies suggest that greater total daily fluid intake may reduce the risk of bladder cancer, perhaps by dilution of carcinogens and promoting chemicals [3]. Acquired genetic alterations in tumor

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suppressor genes and oncogenes play a critical role in bladder cancer initiation and progression [16]. However, there is no clear hereditary pattern and there appears to be only a small increased risk in relatives of bladder cancer patients [25]. There remains a critical need for effective screening as well as primary and tertiary preventive strategies, in addition to continued smoking cessation efforts.

Transitional cell carcinoma is generally classified into two pathways as summarized in Figure 1. The majority present with non-invasive cancers, which encompasses approximately 80% of urothelial carcinomas, with the remainder presenting with invasive disease. The non-invasive disease is usually managed by cystoscopic resection of the tumor, often followed by intravesical cytotoxic and/or immune therapy. Those with superficial disease have a 5-year survival rate of almost 90%, when optimally monitored and treated. However, non-invasive cancer has an approximately 70% chance of recurring, and a 10–20% chance of progressing into invasive disease [26, 27]. Due to the very high probability of recurrence, and risk of ultimately converting to invasive disease, the current standard-of-care mandates frequent urine tests, cystoscopies and resections, which is challenging and costly, yet this may present a unique and attractive opportunity for possible preventive strategies.

The *de novo* invasive form, in contrast, has a far grimmer prognosis with greater than 50% of patients developing metastatic disease and a 5-yr survival of 6% in the metastatic setting [28–30]. Interestingly, these two clinically different scenarios have distinct mechanisms of molecular carcinogenesis. The non-invasive variant is typically a result of gain-of-function mutations of the *ras* or similar growth regulating pathways, including *ras* itself and fibroblast growth factor receptor 3 (FGFR3) [31–33]. In contrast, the invasive variant is most commonly associated with inactivation of tumor suppressor genes, such as p53 and RB [16, 34].

There remains a strong need for the development of effective treatments and preventative strategies to help ameliorate the bladder cancer burden. Furthermore, the development of novel biomarkers of bladder cancer initiation and progression, and monitoring of treatment are also greatly needed.

III. History of Cruciferous Vegetables

The mustard family of plants (Brassicaceae or Cruciferae) have a long history of human cultivation and consumption. It is documented that the Ancient Greeks, Romans, Indians and Chinese all utilized and greatly valued cruciferous vegetables. Interestingly, Brassicaceae crops provide the greatest diversity of crops used by man derived from a single species [35]. The diversity of cauliflower and broccoli-like vegetables from *Brassica oleracea* (wild cabbage) occurred in Europe, probably evolving from germplasm introduced in Roman times from the Eastern Mediterranean. Broccoli (*Brassica oleracea italica)* is a name of Italian origin, coming from the Latin ‘brachium’, meaning an arm or branch and refers to the edible floral shoots on brassica plants, which include cabbages and turnips. It became popular in Northern Europe in the 18th century. Broccoli, as a single main green head (called calabrese and named from the Calabria region of Italy), was introduced in the United States by Italian immigrants in the early 20th century. It is considered a ‘convenience’ vegetable

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and has spread back to Europe from the United States into Japan and other countries in the Pacific Rim over the past 50 years [35]. Today, broccoli is one of the most consumed cruciferous vegetables in the United States, however it pales in comparison to the consumption of the top five fruits and vegetables: lettuce, tomatoes, potatoes, bananas and orange products. For example, the Continuing Survey of Food Intakes estimated 39–42% consume iceberg lettuce and tomatoes, while only 3% consumed broccoli [36]. Worldwide, cruciferous vegetables are commonly consumed and include cabbage, Brussels sprouts, cauliflower, kale, collard greens, arugula (rocket), bok choy, watercress, canola, mustard seeds, radish, daikon, wasabi and horseradish.

Plants from this family are readily distinguished by a cruciform (cross-shaped) corolla, six stamens (the outer two shorter than the inner four), a capsule often with a septum and a pungent watery sap [37]. However, taxonomic classification of Brassicaceae (338 genera and 3709 species) is one of great complexity and controversy. This is because boundaries between species have been poorly delineated, and largely artificially circumscribed until 2006. The use of molecular biology techniques have helped to more clearly define the taxonomy of this important family of plants [37].

IV. Epidemiology of Cruciferous Vegetables, Isothiocyanates and Bladder Cancer

A landmark series of reports from the Health Professional's Follow-Up Study, a prospective cohort epidemiologic study, involving over 47,000 men and published in the late 1990's provided new insight into risk factors for bladder cancer, particularly diet and nutrition [1, 3–5]. Included in these, was a thorough examination of estimated fruit and vegetable intake and the risk of bladder cancer, reporting that intake of cruciferous vegetables, particularly broccoli, had a strong inverse association with bladder cancer risk, with those consuming <1 serving of broccoli/week vs. >1 serving/week associated with a 29% lower risk, while ≥2 servings/week experienced a 39% lower risk (p=.009) [5]. Supporting these findings, a retrospective case-control study of over 1400 participants, from MD Anderson Cancer Center, showed that high ITC intake was associated with 29% decreased risk of bladder cancer and the protective effect was most evident in older individuals and ever- and heavy-smokers. The study also correlated N-acetyltransferase (NAT2) slow acetylators, an enzymatic process involved in carcinogen metabolism, with increased bladder cancer in Caucasians [38]. A recent meta-analysis found that increasing intake of fruits, vegetables, cruciferous vegetables, citrus fruits and fruits and vegetables combined were associated with a statistically significant reduction in bladder cancer risk with similar results observed in a linear dose-response analysis [39]. Furthermore, another meta-analysis including cohort and case-control studies, evaluated the relationship between cruciferous vegetables intake and risk of bladder cancer, finding a significantly decreased risk of bladder cancer in overall cruciferous vegetables intake group and subgroup of case-control studies but not detected in cohort studies [40]. A hospital-based case-controlled study, involving 275 individuals with incident, primary bladder cancer, observed a strong and statistically significant inverse association between bladder cancer risk and raw cruciferous vegetables intake, with the inverse association remaining consistent among current and heavy smokers with three or

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more servings of cruciferous vegetables a month [41]. Furthermore, with an average 8-year follow-up, a strong and significant inverse association was observed between bladder cancer mortality and broccoli intake, particularly raw broccoli intake [42]. Interestingly, in a Multiethnic Cohort Study, it was found that in women, total fruits and vegetables, total vegetables, yellow-orange vegetables and total fruits and citrus fruits were all inversely associated with the risk of invasive bladder cancer in risk factor-adjusted models. However, for men, no association for fruits, vegetables, or nutrients were found, overall; although inverse associations were observed for vegetable intake among current smokers, and in ethnic specific analyses, specifically in Latino men [43].

There is emerging evidence that bladder cancer risk increases significantly in individuals who carry genetic variants of phase II enzymes such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1) [44–47]. Phase II enzymes act as cell protectants by detoxifying against potential carcinogens and oxidants. Interestingly, isothiocyanates found in cruciferous vegetables have been shown to induce phase II enzyme activity and this may in part explain their epidemiologic association with decreased bladder cancer risk [33]. This may explain why high consumption of fruits and vegetables, particularly cruciferous vegetables, is associated with reduced risk of bladder cancer [48–51]. Furthermore, genetic variations in DNA repair genes, including ERCC2, D312N and XPC, have also been shown to be associated with increased bladder cancer risk [52].

Conversely, some epidemiologic studies examining cruciferous vegetables intake and bladder cancer risk, have shown no association of decreased risk [53, 54]. For example, a prospective study from Alpha-Tocopherol Beta-Carotene Cancer Prevention Study did not find an association between total fruits and vegetables intake or cruciferous vegetables intake and bladder cancer risk in smokers [2]. In addition, a Swedish prospective population-based cohort study of 82,002 women and men found no association between total fruits and vegetables or cruciferous vegetables intake [55]. A Meta- Analysis on fruits and vegetables intake and risk of bladder cancer risk also did not see a correlation between cruciferous vegetables and bladder cancer risk, however, an inverse relationship between bladder cancer and green leafy vegetables was seen [56].

Inconsistencies in epidemiologic findings may be related to multiple reasons [57]. Firstly, intakes from diet assessment tools cannot capture many variables that may significantly contribute to the potentially anticancer activity of bioactive in fruits and vegetables. The many types of cruciferous vegetables and their multitude of genetic strains will show a diverse array of bioactives. In addition, cooking methods may impact stability, absorption and bioavailability. The context of the meal where cruciferous vegetables are consumed may also impact host exposure to bioactives [58, 59]. In recent years, many studies are showing exceptionally diverse pharmacokinetics of phytochemicals in humans as host genetics impact the metabolism and degradation of phytochemicals [60–62]. Furthermore, epidemiologic studies must consider the exposures to known bladder carcinogens as critical variables and examine key interactions with diet and host genetic polymorphisms associated with carcinogen metabolism. Overall, in spite of the recognized caveats, the epidemiologic findings support a hypothesis that cruciferous vegetable intake is related to decreased risk of bladder cancer. Further well-designed prospective studies addressing the interactive risk

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factors are needed to explore the potential protective impact of cruciferous vegetables over the life cycle of bladder carcinogenesis [40]. In addition to human studies, one logical and necessary step is to test these hypotheses in relevant pre-clinical bladder cancer models of superficial and invasive disease.

V. Chemistry, Bioavailability and Metabolism of Cruciferous Vegetable Isothiocyanates

A remarkable characteristic of cruciferous plants is their high content of glucosinolates, which often approaches greater than 1% of their dry weight [63]. These compounds serve an important evolutionary protective role as they are a component of the myrosinase system where myrosinase enzymes are sequestered in the intact plant separately from glucosinolate substrates. When the plant is damaged, by an insect or by human chewing, chopping or digestion for example, myrosinase catalyzes the conversion of inactive glucosinolate precursors to an unstable intermediate, which rearranges to produce a nitrile, thiocyanate, or isothiocyanate that protect the plant as illustrated in Figure 2. Isothiocyanates have been shown to have broad antibiotic properties including antimicrobial, nematocidal, antifungal and antiprotozoal [64]. They also cause specific positive and negative feeding cues for some insects, can exhibit insecticidal properties as well as allelopathy, where they can suppress the growth of neighboring plants [63]. Diverse glucosinolates and their corresponding isothiocyanates are common among cruciferous vegetables, with the glucosinolate backbone demonstrating over 100 different R groups [65] and classified as alkyl, indolyl, or benzyl derivatives. Isothiocyanates are often volatile adding spiciness to food products such as horse radish, wasabi, radishes, and many cruciferous sprouts such as those of broccoli. An emerging body of literature involves the bioactivity and fate of indolyl glucosinolates in humans. Downstream metabolites, indole-3-carbinol and di-indolyl methane, are well-studied in this realm however, this review’s focus is on the alkyl isothiocyanates sulforaphane and erucin which have established efficacy in many experimental models of cancer including of the bladder.

The glucosinolate content of cruciferous plants varies greatly, influenced by a multitude of factors including the region, season, climactic conditions of the specific year grown, length and mode of storage and soil conditions among many other factors. However, genetics have been shown to have a greater effect on glucosinolate content over environment [66–68]. Previous damage to a plant primes for higher production of glucosinolates as a protective mechanism, watering also increases overall levels [69]. Contrastingly, lower growing temperatures, with plants harvested in the winter or autumn versus the spring or summer as well as selenium-enriched soil, causes decreased plant glucosinolate levels [67, 68]. Young broccoli plants, especially seeds and sprouts, have 20–50 times the levels of glucosinolates than more mature market-stage plants [70]. Since glucosinolates serve in plant defense, the myrosinase system has evolved to be most potent in immature organs to protect a plant until it reaches its reproductive stage.

Plant tissue damage usually induces glucosinolate hydrolysis to isothiocyanates and subsequent isothiocyanate losses if vegetables are not immediately consumed. Many food

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handling practices employed for storage and preparation are disruptive. When vegetables are cooked, glucosinolates can be reduced by 30–60% via thermal degradation but primarily due to leaching into the cooking water when boiled [71–73]. Thawing of frozen cruciferous vegetables, without previous inactivation of myrosinase, leads to almost complete loss of glucosinolate as the cell structure is broken and water becomes available for reactions to occur [74]. Commercial transport, distribution and storage during the retail sale period also lead to significant losses, with a 40% loss of aliphatic glucosinolates after 7 days of storage and a 66% loss after 10 days [74]. To minimize these losses, rapid cooking at sub-boiling temperatures and with scant cooking water – e.g. sautéing, steaming, or stir-frying - thermally inactivates myrosinase without leaching and thereby stabilizes glucosinolate levels. Greater isothiocyanate yields at consumption can be realized by pre-treating vegetables at 60 °C to selectively inactivate epithiospecifier protein (ESP) such that a greater proportion of intermediates rearrange to isothiocyanates upon processing or chewing [74–76]. It has also been shown that isothiocyanates in broccoli juice are relatively thermolabile and pressure stable [77]. In addition, High Pressure Processing (HPP) caused no negative effects on the glucosinolate-myrosinase system in broccoli sprouts [78].

The bioavailability and metabolism of vegetables isothiocyanates is a key issue when considering the potential impact these compounds may have on human health. Glucosinolates have limited bioavailability, yet when converted to isothiocyanates, metabolites are recovered in biological samples. Collectively, published data in rats and humans suggests that isothiocyanates from fresh cruciferous vegetables can reach μM concentrations in the blood, accumulate in tissues and persist with a half-life of approximately 2 hours [79]. After conversion by plant myrosinase, isothiocyanates can be transported from the gastrointestinal tract by passive, facilitated or active transport [73]. The high activity of myrosinase in fresh cruciferous vegetables rapidly produces isothiocyanates during chewing and isothiocyanate metabolites can reach their maximum concentration in blood within 30 minutes [80, 81]. During uptake by enterocytes or once they reach the blood and liver, isothiocyanates which are reactive electrophiles and usually trapped and further metabolized. A major first step of metabolism is by conjugation to glutathione (GSH) by Glutathione S-Transferase (GST) enzymes or by direct reaction with GSH or cysteine since thiol groups are strong nucleophiles. Consequently human genetic polymorphisms in GSTs were expected to be one contributing factor impacting isothiocyanate metabolite concentrations *in vivo* yet were not terribly predictive of recoveries [80, 82, 83]. Isothiocyanates conjugated to GSH are processed by the mercapturic acid pathway leading eventually to N-acetylcysteine (NAC) conjugates which are the major form present in urine, as depicted in Figure 3. Acetylated isothiocyanates have demonstrated effects on histone acetylation as histone deacetylases inhibitors. Although NAC-isothiocyanate conjugates are more stable than isothiocyanates themselves, NAC conjugates exist in an equilibrium with free isothiocyanates and the reversible nature of these bonds [84] might explain in part how conjugated isothiocyanates continue to influence biological systems. Free isothiocyanates can also react with protein thiols on the cysteine-rich protein, KEAP-1 thus modulating the KEAP1-Nrf2-antioxidant response element (ARE) signaling pathway [85] where several cysteine residues were reversibly modified through conjugation to sulforaphane.

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If myrosinase in foods is inactivated, glucosinolates reach the large intestine where they can be degraded by the resident microflora if they express the requisite myrosinase activity [86]. In a study by Clarke et al. [87] isothiocyanate metabolite levels in humans were measured after delivering a glucosinolate supplement devoid of myrosinase activity and compared to matched amounts of glucosinolates with high levels of endogenous plant myrosinase. Isothiocyanate bioavailability was ~40–80 fold higher in urine of volunteers when consumed as fresh broccoli sprouts [87]. More recently, researchers have aimed to encourage the growth of bacteria in the lower gut with myrosinase activity and thereby enhance conversion to isothiocyanates when humans ingest cooked cruciferous vegetables [88]. This is a promising strategy to maximize cruciferous vegetables health benefits since cruciferous vegetables are often preserved through cooking making isothiocyanate exposure reliant on the gut microbiome.

Two of the key isothiocyanates showing potent bioactivity in a variety of chemoprevention studies are sulforaphane (SFN) and erucin (ECN) which both reach the bladder via the urine after consumption of cruciferous vegetables. This has been shown *in vivo* where rats were fed purified glucosinolates. SFN and ECN conjugates as well as free SFN and ECN were found in the urine [89]. In addition, a study performed in over 18,000 men with colorectal cancer showed that cruciferous vegetable consumption, in single-void urine samples, led to urinary isothiocyanates concentrations that averaged 2.75 μmol/g creatinine, where urinary ITC concentrations were expressed in units of urinary creatinine to account for varying water contents of urine samples [90]. Cumulative excretion of isothiocyanates in urine have been shown to be about 50 times higher than the maximum concentration in the plasma, making the feasibility of isothiocyanate chemoprevention particularly powerful in the bladder [91]. Interestingly, SFN and ECN represent redox partners of one another (sulfinyl and thio-ether, respectively) and both are present in broccoli sprouts. When SFN and ECN were administered individually to mice, metabolites of both SFN and ECN were detected in biological samples [76, 92] demonstrating interconversion between the two forms. In studies by Clarke et al. [87, 93] it was found that between individuals there was great variance in the ratio of urinary ECN/SFN metabolites and these differences were reproducible over time. In the mouse studies this ratio was similar whether SFN or ECN was incorporated into chow, suggesting a steady state ratio. The varied human ECN/SFN ratios are provocative and may be predictive of individual biological impacts of isothiocyanate exposures. If so, elucidating the biochemical basis would be of great value to steer ratios towards optimal benefits.

More recently an analogous type of alkyl isothiocyanate with similar phase II enzyme-inducing activity and chemopreventive promise has come to the forefront. Glucoraphenin and glucoraphasatin occur in radish roots, sprouts, and seeds and upon hydrolysis are converted to sulforaphane and raphasatin. They share the same structure with sulforaphane and erucin except contain a double bond at the 3-position of the alkyl chain. The two isothiocyanates occur with similar abundance to one another and in comparisons with broccoli sprouts for bioactivity, radish sprouts are similarly potent. Also of interest is that radishes are mostly devoid of ESP and thus generate higher yields of isothiocyanate without special pre-treatments [94–96].

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Human Studies in Isothiocyanate Metabolism

Several small-scale clinical studies have been reported examining the safety, tolerance and metabolism of isothiocyanates. A randomized, placebo-controlled, double-blind Phase I clinical trial of healthy volunteers used 25 μmol or 100 μmol of glucosinolates or 25 μmol of pure isothiocyanate (which is equivalent to about 10 g of dried broccoli or 1 g of dried broccoli sprouts) for 7 days showed no significant toxicities and much higher and more consistent excretion of isothiocyanates in the cohort fed isothiocyanates directly compared to those given glucosinolates [97]. A study comparing administration of a broccoli sprout derived sulforaphane-rich drink (SFR) vs. a glucoraphanin rich drink (GRR) found that the SFR led to fastest and highest peak concentration of serum SFN, while the GRR had considerable slower elimination rates of SFN, therefore implying that the optimal formulation would be a combination of SFR and GRR to achieve peak concentration for activation of targets as well as prolonged inhibition of other protective actions of SFN [98]. Healthy human volunteers given 200 μmol of broccoli sprout isothiocyanates exhibited peak plasma concentrations of 0.94–2.27 μmol/L at 1 hour and declined with first order kinetics with a half-life = 1.77 ± 0.13h [81]. Furthermore, raw broccoli was shown to result in faster absorption, higher bioavailability and higher peak plasma concentrations in human volunteers over cooked broccoli [99].

Interestingly, smokers consuming 250 g/day of steamed broccoli showed 41% decreased levels of oxidized DNA lesions and 23% increased resistance to H_2O_2 induced DNA strand breaks in peripheral blood mononuclear cells [100]. Another study looked at eight healthy women undergoing reduction mammoplasty who were given a single dose of broccoli sprout preparation containing 200 μmol of SFN. Following oral dosing, SFN metabolites were readily measurable in human breast tissue and were enriched in the epithelium [101]. One study conducted in China, reported striking inter- individual differences in the bioavailability of broccoli sprout isothiocyanates, an important consideration in the design of future human clinical trials [102]. Overall, the current literature supports the oral bioavailability of isothiocyanates, producing biologically active plasma and urine concentrations after consumption of broccoli, sprouts and pure isothiocyanates. Yet, there is clearly a need for further carefully controlled and powered clinical studies examining metabolism, bioavailability and safety of isothiocyanates. In addition, studies documenting the bioactivity of these compounds in relation to modulation of bladder carcinogenesis are also imperative.

VI. *In Vitro* and *In Vivo* Studies of Isothiocyanates and Bladder Cancer

There are limited *in vitro* and *in vivo* experimental studies examining at the potential inhibitory effects of cruciferous vegetables or their components on bladder carcinogenesis. In addition, there is a need for more *in vivo* bioavailability and metabolism studies of isothiocyanates to determine if they can reach appreciable plasma concentrations and reach important target organs and tissues progressing through the carcinogenesis cascade. Furthermore, addition studies focusing upon cellular targets mediating critical steps in bladder carcinogenesis are needed.

The ability of phytochemicals from food to inhibit carcinogenesis began over 5 decades ago, where the observation that animals fed a complex diet were at a lesser risk of developing

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cancer than those consuming a semi-purified diet [103]. The two diets were nutritionally comparable in regard to major nutrients but differed significantly in the amount of non-nutrients they contained. Dr. Lee Wattenberg subsequently fed a semi-purified diet and incorporated single food components one at a time to determine which of these exhibits an anticancer effect. He found that cruciferous vegetables are partly responsible for the observed anti-cancer effect of a chow diet [104]. In 1992, Paul Talalay reported the isothiocyanate sulforaphane to be a potent inducer of phase II enzyme activity and suggested that it may be responsible for the anti-cancer effects seen with broccoli consumption [105, 106]. Subsequently, several *in vitro* and *in vivo* studies utilizing broccoli and broccoli sprout extracts as well as pure isothiocyanates have been performed looking at their effects in multiple cancers including lung, esophageal, prostate, breast and colorectal among several others [106–111]. However, the literature on bladder cancer and cruciferous vegetables is much more limited. *In vitro*, human bladder carcinoma cells (UMUC3 and T24) were significantly inhibited by isothiocyanates at doses of 7.5–30 μM, leading to induction of apoptosis and arrested cell cycle progression in the G2/M and S phases [112]. Our data also shows induction of apoptosis and cell cycle arrest in cell lines, ranging from superficial to invasive cell lines, treated with sulforaphane or erucin, with less toxic effects on normal human urothelial cells [92].

There are few *in vivo* bladder cancer studies utilizing isothiocyanates. In a long term (36 week) rat study, animals fed 160 μmol/kg bw/day broccoli sprout extract and given 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), a specific bladder carcinogen, lead to a significant decrease in incidence, multiplicity, size and progression of bladder cancer. This was attributed to the broccoli extract's ability to significantly induce glutathione-S-transferase and NAD(P)H:quinine oxidoreductase 1, enzymes that detoxify oxidants and carcinogens [113]. Another study showed that sulforaphane can inhibit 4-aminobiphenyl-induced DNA damage in RT4 cells and in bladder tissue [114]. It has also been shown that allyl isothiocyanate rich mustard seed powder (71.5 mg/kg giving from a singirin dose of 9 μnmol/kg) can inhibit bladder cancer growth and block muscle invasion by 34.5% in an orthotopic rat bladder cancer model [115]. Also, in a study of n-butyl-(4-hydroxybutyl)nitrosamine (BHBN) induced bladder tumors in rats, both 2-mercaptoethane sulfonate (MESNA) and 6-phenylhexyl isothiocyanate inhibited tumorigenesis, but phenethyl isothiocyanate did not [116]. We have also shown the broccoli isothiocyanates have the ability to inhibit established bladder cancer, utilizing a UMUC3 subcutaneous xenograft tumor model [92]. Additional cellular, animal and preclinical studies are described in a recent review on cruciferous vegetables, isothiocyanates and the prevention of bladder cancer [117].

Potential Toxicity of Isothiocyanates

Although isothiocyanates have been shown to exhibit anti-bladder cancer effects, they have also been shown to potential toxic effects. One of the major toxic effects of isothiocyanates involves their effects on thyroid function, interfering with iodine uptake and thyroid hormone synthesis and leading to hypothyroidism and goiter (enlargement of the thyroid gland) [97, 118, 119]. An inhibition of proper thyroid function may impact metabolism in almost all tissues, including effects on reproductive organs. In studies looking at animal feed

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rich in cruciferous vegetables, a reduction in fertility of male and female animals, as well as growth reduction, and reduction in milk and egg production have been observed [120]. Furthermore, glucosinolate hydrolysis may cause irritation of gastro-intestinal mucosa, which may lead to local necrosis and hepatotoxicity [121].

There have even been studies that have linked isothiocyanates, in low concentrations, with compromising the function of immune cells and impairing genome stability [122]. Isothiocyanates are extremely electrophilic and have the ability to bind sulphydryl groups of biologically important molecules, contributing to their potential toxicity. In fact, some isothiocyanates have also been shown to have bladder cancer inducing properties, particularly phenethyl isothiocyanate (PEITC) and benzyl isothiocyanate (BITC). In rats fed a diet including 0.1% PEITC for 45 weeks, 92% of the mice formed bladder carcinomas, which remained even when the phenethyl isothiocyanate diet was discontinued [123]. Both pre- and post- initiation events were studied. Pre-initiation, 0.1% PIETC or BITC for 14 days lead to increased inflammatory cell infiltration and hyperplasia of the bladder epithelium [124]. In addition, post-initiation effects of 0.1 % PEITC and BITC were examined in urinary bladder carcinogenesis with or without pre-treatment with diethylnitrosamine (DEN) and BBN, and both isothiocyanates showed strong promoter and some complete carcinogenic potential of bladder cancer [125]. One possible reason for this observation could be the accumulated dose of urinary isothiocyanates with this dosing regimen. It is estimated that NAC-ITCs could have been constantly maintained at levels far greater than 1 mmol/L and that although isothiocyanates can be beneficial at preventing or inhibiting bladder cancer growth at lower doses, at excessively higher doses, they may be harmful [112]. In addition, it is possible that the benzyl or phenethyl isothiocyanates may have some harmful effects, but this has not been reported for other isothiocyanates such as sulforaphane or erucin. Broccoli and broccoli sprouts do not have appreciable concentrations of these isothiocyanates and may be a better option over cruciferous vegetables containing higher levels of PIETC or BITC such as cauliflower, cabbage or Brussels sprouts, especially if these compounds are used in high doses for prevention or treatment of bladder cancer [126].

Toxicity can be explained by isothiocyanates ability to readily accumulate in bladder tissue. Given a rat that would consume an average of 16 g of food/day with a diet containing 0.1% isothiocyanate and produces 30 ml of urine in 24 hours, the rat will consume ~100 μmol isothiocyanate daily [127]. This would lead to a urinary concentration of ~1900 μM in the bladder which is much higher than can be achieved by human consumption and may explain the toxicity observed. Furthermore, cell culture studies have shown that only low concentrations of 5–20 μM are necessary to inhibit the growth of cancer cells further supporting the idea that large doses of isothiocyanates are not necessary [112, 128]. However, because other organs do not accumulate isothiocyanates and cannot reach such high concentrations as the bladder, toxicity is a possibility when attempting to achieve high enough concentrations for chemopreventive effects in these organs. Overall cruciferous plants at usual intake appear to be safe in humans [97] with the exception of allergies and with special precaution taken by those taking blood-thinning medications such as Warfarin [129].

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VII. Mechanisms of Action of Isothiocyanates and Bladder Cancer

A wide body of evidence suggests that isothiocyanates possess potent anti-cancer activity via induction of apoptosis through several distinct molecular mechanisms of action that include targeting the Keap-1- and Nrf2-dependent adaptive stress response, inhibition of phase I enzymes (i.e. cytochrome p450), interfering with inflammatory and pro-growth/-survival intracellular signaling pathways, and through disruption of mitochondrial function, reviewed extensively in [130, 131].

Modulation of Carcinogen Metabolizing Enzymes

A well-defined isothiocyanate anti-cancer mechanism of action, which may modulate bladder cancer initiation, involves inhibition of phase I enzymes (i.e. cytochrome p450) and the induction of phase II enzymes, such as glutathione-S transferase (GST), quinone reductase (QR), and NAD(P)H:quinone oxidoreductase-1 (NOQ1), reviewed in [131]. Phase I enzymes cause carcinogenic compounds to become more hydrophilic through numerous oxidation, reduction, and hydrolysis reactions, causing increased reactivity and DNA damage [132, 133]. Furthermore, certain cytochrome p450 enzymes are associated with an increased risk of bladder cancer [127]. In contrast, phase II enzymes cause conjugation of the reactive intermediates, rendering them more water-soluble and therefore excreted in the urine through the mercapturic acid pathway [132, 133]. GSH is regulated by phase II enzymes, and is a principle ligand that binds with electrophiles and reactive oxygen species (ROS) [132].

Isothiocyanates have also been demonstrated to interact with the bladder epithelium and induce GST and NQO1, well-known cytoprotective enzymes capable of detoxifying carcinogens [134]. Interestingly, NQ01 has also been shown to stabilize the tumor suppressor p53 [135]. The bladder was also shown to be one of the most responsive tissues for the induction of these enzymes by broccoli sprout extracts which could especially be effective in protecting the bladder against cancer initiation [33]. Isothiocyanates appear to induce phase II enzymes at the transcription level, through the antioxidant response element (ARE) in the 5’-upstream region of mRNA [132, 136, 137]. This response element is activated through the binding of nuclear factor E2-related factor 2 (Nrf2), which can be regulated through mitogen activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and other kinases and phase II enzyme inducers [132, 136]. When cytosolic Nrf2 is released from its chaperone, it translocates to the nucleus to induce the transcription of phase II enzymes [132]. Since induction occurs at the level of transcription, chemoprevention through this mechanism of action is thought to be more effective prior to the presence of the carcinogen.

Modulation of Cell Cycle and Apoptosis

Evidence indicates isothiocyanates may enhance the deletion of initiated cells from damaged tissue through inducing programmed cell death. One way in which isothiocyanates have been shown to do this is through modulation of the cell cycle. Invasive bladder cancer cells, treated with sulforaphane, have been shown to undergo cell cycle arrest at the G2-M and S phases [138]. In addition, isothiocyanates have been shown to enhance sensitivity to

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apoptosis. Sulforaphane (SFN) has also been shown to induce caspase-mediated (caspase 8 and 9) apoptosis in both bladder and prostate cancer cells, as well as cause the overexpression of Bax and down-regulation of Bcl-2 [139]. We have shown that broccoli isothiocyanates, sulforaphane and erucin, can significantly induce apoptosis in superficial (RT4) and invasive (J82, UMUC3) human bladder cancer cell lines, by induction of caspase 3/7 activity and PARP cleavage. We have also shown this phenomenon *in vivo* in a UMUC3 subcutaneous xenograft model, where apoptosis was induced, revealed by an increase in PARP cleavage when mice were treated with broccoli sprout diet. Furthermore, we revealed RT4, J82 and UMUC3 cells to accumulate in the G2/M phase of the cell cycle when treated with either SFN or ECN that was regulated via suppressed levels of survivin, epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2/neu) [92].

A previous study by Savio, et. al. revealed that allyl isothiocyanate (AITC) (mustard cell essential oil) casued cell cycle arrest, increased apoptosis rates, and varying genotoxicity, which was dependent on the mutational status of TP53 [140]. Savio and colleagues also showed that while AITC affected the BAX/BCL2 pathway in RT4 cells (wt TP53), AITC affected the cytokinesis related ANLN and S100P in T24 (mutant TP53) cells. This suggests AITC regulates bladder cancer cell gene expression in a TP53 genotype-dependent manner [141].

Isothiocyanates have been shown to bind to microtubules to ultimately induce cell cycle arrest and apoptosis. A study by Overby, et. al. showed that isothiocyanates disrupted microtubules in *Arabodopsis thalinia* and in AY-27 rat bladder cancer cells suggesting that plant and mammalian cells share isothiocyante-mediated anti-growth mechanisms [142].

Additionally, SFN was also shown to induce mitotic arrest, however, not in the G2 phase of the cell cycle. Park, et. al. revealed that 5637 human bladder cancer cells treated with SFN underwent caspase-dependent apoptosis via accumulation of reactive oxygen species (ROS) [143]. Mechanistically, Jo and colleagues showed that SFN treated T24 human bladder cancer cells underwent apoptosis via the mitochondria-mediated intrinsic pathway with concomitant elevation of ROS, and activation of endoplasmic reticulum stress (ER) and the Nrf2 signaling pathway suggesting that ER and Nrf2 may represent targets for SFN-dependent apoptosis [144]. Another report revealed that SFN not only affected the epithelial-to-mesenchymal transition but also targeted both the OX-2/MMP2,9/ZEB1, Snail and miR-200c/ZEB signaling pathways in T24 bladder cancer cells [145].

In addition to SFN acting as a single therapeutic, Islam and colleagues showed that SFN in combination with the carbonic anhydrase inhibitor acetazolamide (AZ) targeted both the pH homeostasis pathway and the PI3K/Akt intracellular signaling pro-survival pathway allowing for increased anti-tumor efficacy *in vitro* and *in vivo* [146]. SFN+AZ combination treatment allowed for down-regulation of components of pH homeostasis related carbonic anhydrase 9 (CA9), E-cadherin, N-cadherin, and vimentin proteins while diminishing the epithelial-to-mesenchymal transition and decreased PI3k/Akt survival signaling suggesting a potential link between pH homeostasis and pro-survival signaling [146]. Another previous report showed that SFN inhibited proliferation of BIU87 bladder cancer cells via

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upregulation of IGFBP-3 expression levels and negatively regulated nuclear factor-κB (NF-κB) signaling [147]. Collectively, these findings suggest that isothiocyanates, specifically SFN targets multiple pathways in bladder cancer cells including the intrinsic apoptotic and pro-growth/-survival intracellular signaling pathways to promote apoptotic cell death.

In addition to apoptotic induction in bladder cancer cells through modulation of the adaptive stress response, phase I/II enzymes, pro-growth/-survival signaling, and the intrinsic apoptotic pathway, SFN has also been shown to be effective in inhibiting all essential steps of cancer vessel formation from proangiogenic signaling to endothelial cell proliferation, migration and tube formation [148, 149]. Furthermore, SFN has been shown to inhibit cancer cell metastasis. Utilizing B16F-10 melanoma cells, which are highly metastatic to the lungs, when injected through the mouse tail vein, SFN was shown to reduce metastasis, by inhibiting matrix metalloproteinases (MMPs). MMPs are proteases capable of degrading the extracellular matrix, and thereby promoting metastasis [150]. Together, these findings suggest that isothiocyanates are compounds with potent anti-tumor effects with several distinct mechanisms of action that inhibit proliferation, and induce apoptosis and cell cycle arrest.

Epigenetic Modulation

There is emerging evidence that broccoli isothiocyanates modulate epigenetic activity, partly through microRNA (miR) regulation and act as potent inhibitors of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs), reviewed in [151]. The classical central causes of cancer revolve around changes in DNA structure leading to cell growth deregulation. However, the emerging field of epigenetics reveals that histone changes can also lead to cancer progression, through opening or closing regions of oncogenes or tumor suppressor genes, respectively [152–155]. HDAC activity has been shown to occur in many cancers, leading to deregulation of differentiation, cell cycle, and apoptosis. The tumor suppressor p21 appears to be a target of HDACs and is ‘silenced’, however, when HDAC inhibitors are utilized, the p21 gene remains open and inhibits cancer progression [156]. Sulforaphane has been shown to exhibit HDAC inhibitory activity in prostate cancer cells *in vitro* [157]. *In vivo,* a xenograft model with human prostate cancer cells also exhibited HDAC inhibition, when mice were fed SFN [158]. HDAC inhibition by SFN has also been shown in humans fed broccoli sprouts, where peripheral blood mononuclear cells were isolated and HDAC inhibitory activity was assessed [159]. Our laboratory has recently shown that both isothiocyantes SFN and ECN have the ability to significantly inhibit HDAC activity as well as histone acetyltransferase (HAT) activity, leading to small increased levels in acetylated histone H3 [160]. Furthermore, we have shown a potential novel epigenetic mechanism of broccoli isothiocyanate action, whereby histone phosphorylation decreased with concomitant increases in PP1β and PP2A phosphatase activity in the presence of SFN and ECN treatment [160], where we have previously identified increased histone phosphorylation as a biomarker of bladder cancer progression [161]. Taken together with evidence in the literature, our findings suggest that isothiocyanates act as effective epigenetic modifiers both as HDAC inhibitors and now as HAT inhibitors and phosphatase enhancers to ultimately affect the novel biomarker, histone H1 phosphorylation, to induce anti-tumor activity. To summarize, isothiocyanates have tremendous potential both as therapeutic and

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preventative compounds due to their ability to target multiple pathways with numerous mechanisms of action in bladder cancer including the adaptive stress response, phase I/II enzyme modulation, pro-growth/-survival/-inflammatory signaling, the intrinsic apoptotic pathway, and epigenetic modulation.

VIII. Future Approaches

One potential challenge in the field of isothiocyanates as therapeutic and preventative agents for bladder cancer is targeting the compounds specifically to tumor cells, while leaving healthy tissue unharmed. One novel therapeutic approach is via the emerging fields of nanotechnology and nanomedicine for targeted and/or enhanced drug delivery. A recent report demonstrated the ability to construct rhodamine B isothiocyanate-labelled polyacrylic acid-coated cobalt ferrite nanoparticles (NPs) that were endocytosed with significantly greater efficacy by cancer urothelial cells compared to normal urothelial cells suggesting a selective uptake mechanism by cancerous urothelial cells [162]. In addition, Zhang, et. al. built several versions of β-cyclodextrin functionalized mesoporous silica NPs with hydroxyl, amino, and thiol groups and tested mucoadhesitivity properties on urothelium and found that thiol-modified NPs bound to a significantly higher degree compared to hydroxyl and amino modified NPs [163]. Furthermore, doxorubicin-loaded thiol-modified NPs were shown to undergo sustained drug release upon acidification [163]. Collectively, these findings represent a promising and exciting novel area of bladder cancer research that merges tumor biology and nanotechnology.

A deeper understanding of potential mechanisms of action may help us define better strategies for prevention and therapy by cruciferous vegetables or their components. The future integration of genomics, transcriptomics, proteomics and metabolomics, called systems biology, will offer deeper insights into the study of both bladder carcinogenesis as well as the cruciferous vegetable family. For example, these tools may identify new biomarkers in urine or other samples to effectively diagnose bladder cancer earlier and further define subtypes for study. A recent publication shows a pattern of fourteen metabolites including lactic acid, leucine, valine, phenylalanine, glutamate, histidine, aspartic acid, tyrosine, serine, uracil, hypoxanthine, carnitine, pyruvic acid and citric acid linked to early bladder carcinogenesis [164]. Furthermore, metabolomics has been used to study how growing conditions, such as light exposure, can ultimately change the nutritional and phytochemical value, and ultimately the cancer fighting abilities, of broccoli sprouts [165]. Metabolomics has additionally been used to help identify metabolite and transcript biomarkers, which could be useful in the cultivation of broccoli and other *Brassica* vegetables for increased insect resistance [166]. Fascinatingly, underlying mechanisms and key molecular targets involved in the ability of cruciferous vegetables to improve human health have been characterized through plasma metabolite profiles before and after human consumption of broccoli sprouts. The investigation identified several potential molecular targets of crucifers, including fatty acids, glutathione, glutamine, cysteine, dehydroepiandrosterone and deoxyuridine monophosphate, aiding in the study of established and emerging health benefits that the consumption of cruciferous vegetables may possess [167]. It is apparent that future research utilizing metabolomics and its integration with

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additional –omics technologies has strong potential of helping both the fields of bladder cancer and isothiocyanates significantly advance.

IX. Concluding Thoughts

The natural history and pathophysiology of bladder cancer and the current standard-of-care surveillance programs allow for several opportunities to utilize preventive and treatment strategies. The anatomy of the bladder allows cancers at this site to be monitored easily through outpatient cystoscopy, urine cytology, and novel biomarker-based urine tests. Principally, the bladder is a storage compartment for urine, which provides a route of exposure for excreted carcinogens, as suggested by the strong association between bladder cancer risk and exposure to environmental carcinogens, such as tobacco [168]. However, this can also be exploited by enhancing concentrations of anti-carcinogenic compounds, for example from the diet, for prevention. As we reflect upon several decades of cancer prevention efforts, we have seen major accomplishments [169–176]. Yet, we face enormous challenges as a discipline/field with several large high profile human chemoprevention studies showing no benefit [177–179]. We firmly believe that successful human prevention studies are those with the strongest portfolio of supportive preclinical data, particularly with *in vivo* experimental models. Thus, additional preclinical studies examining multiple formulations of cruciferous vegetables relevant to human application are needed in a variety of modern models of bladder carcinogenesis [180]. Furthermore, critical human clinical trials utilizing isothiocyanate rich food products, extracts, concentrates or chemically pure phytochemicals are needed to examine bioavailability, safety, and biomarkers of impact in bladder carcinogenesis and other cancers.

Through the performance of well-designed rodent and human investigations, we have the ability to determine the stages of bladder carcinogenesis where cruciferous vegetables may impact risk. If these foods or compounds do indeed prove to demonstrate clear and potent bioactivity, they can be utilized to improve upon the bladder cancer burden in several ways. One strategy would be “primary” chemoprevention by targeting high-risk populations such as heavy smokers and those with environmental/occupational exposures [181]. Another strategy is a “tertiary” chemopreventive approach, in patients who had a successful complete cystoscopic resection of their superficial bladder cancer. In this case, the goal is to reduce or eliminate the well-known high risk of recurrent tumors and the progression to invasive disease. In addition, patients with diagnosed invasive cancers and scheduled for cystectomy could be fed well-characterized isothiocyanate-rich food products in the period between diagnosis and surgery in order to examine the dietary impact on molecular and cellular events in an established cancer. Furthermore, data for additive or synergistic effects are necessary.

Finally, it is also conceivable to combine dietary or phytochemical interventions with established and emerging treatments for bladder cancer, such as immunotherapy, chemotherapy, and radiation, although additional preclinical evidence and supportive mechanistic studies are needed. While foods alone are unlikely to have therapeutic effects on high grade and aggressive cancers, particularly those in the metastatic state, it is conceivable that foods may enhance the activity of other therapeutic agents when provided in novel

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combinations or sequence. Based upon our work and others', we believe that the use of cruciferous vegetable consumption, or the development of specific food products rich in cruciferous vegetable components, warrants additional studies in bladder cancer prevention as well as part of future effective treatment strategies. The bladder offers multiple opportunities for intervention with foods or phytochemicals in cancer prevention and it is up to us to best harness these opportunities.

Acknowledgements

C.R.L is a recipient of a National Institutes of Health T32 Award in Oncology Training Fellowship at The Ohio State University Comprehensive Cancer Center, T32 CA009338. This publication was made possible through support from Foods for Health, a focus area of the Discovery Themes initiative at The Ohio State University.

List of Abbreviations:

ITC	Isothiocyanate
GLU	Glucosinolate
SFN	Sulforaphane
ECN, ERN	Erucin
GSH	Glutathione
GST	Glutathione S-Transferase
NAC	N-acetylcysteine
PEITC	phenethyl isothiocyanate
BITC	benzyl isothiocyanate
Nrf2	nuclear factor E2-related factor 2
PI3K	phosphatidylinositol 3-kinase
AITC	allyl isothiocyanate
HDAC	histone deacetylase
HAT	histone acetyltransferase

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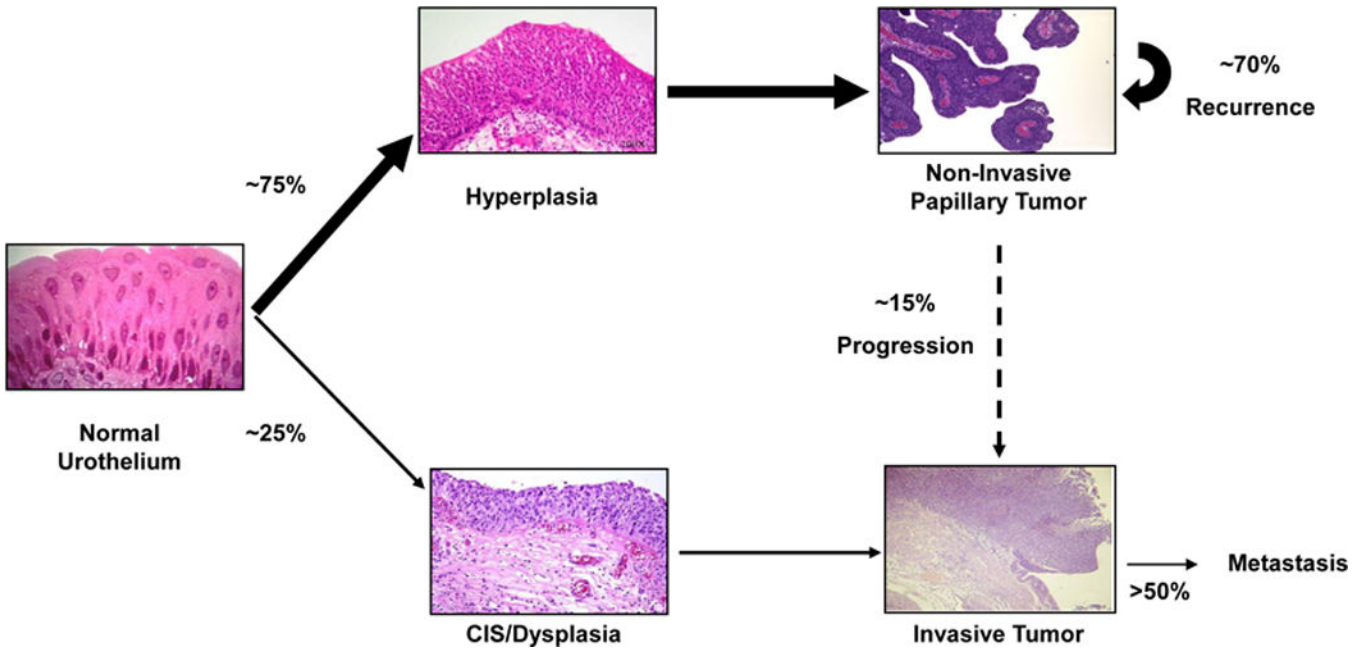

Figure 1. Transitional Cell Carcinoma Pathogenesis.

Transitional Cell Carcinoma of the urinary bladder tends to follow two pathways of pathogenesis. The more common non-invasive papillary tumor pathway has a high survival rate, but a high chance of recurrence, and a chance of progression. The invasive tumor pathway has a very high likelihood of developing into metastatic disease and a much lower rate of survival than the non-invasive tumor. *Images, 'unpublished findings'.*

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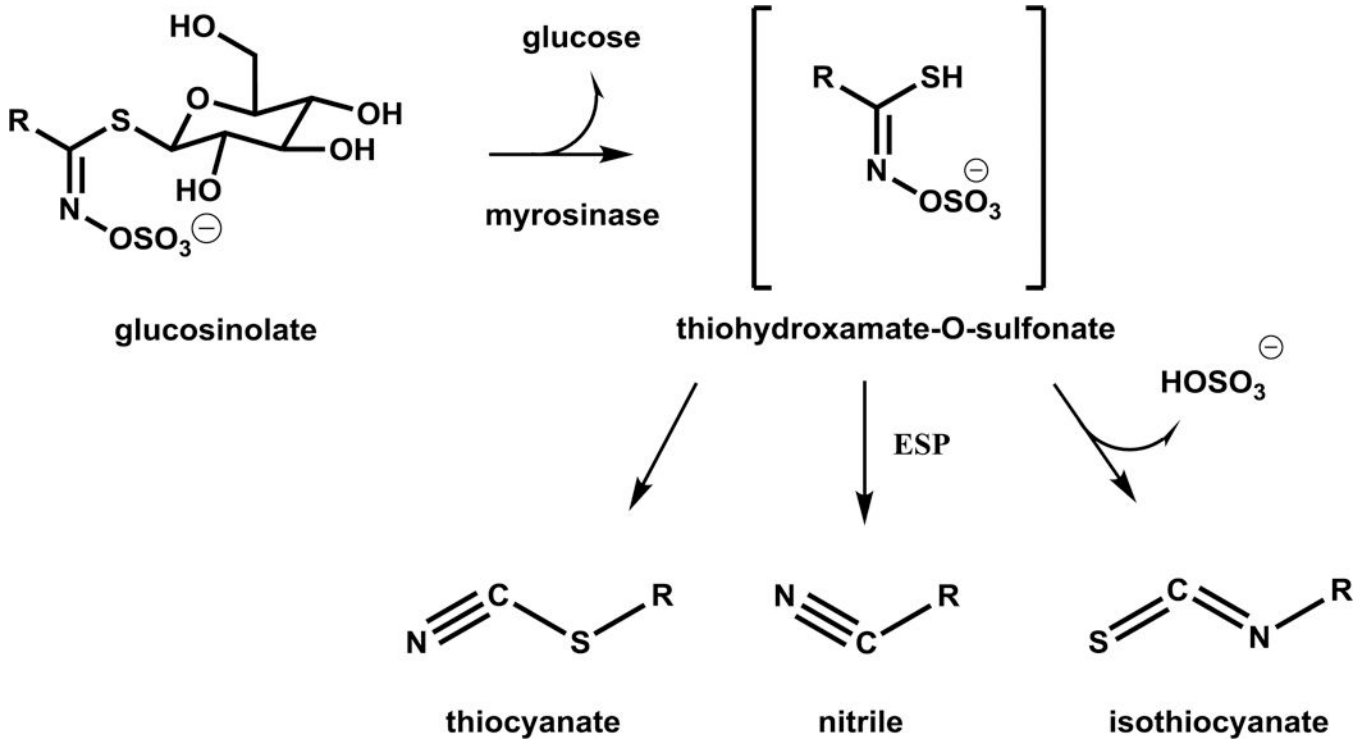

Figure 2. Glucosinolate to isothiocyanate conversion.

Glucosinolate (GLU) hydrolysis mediated by myrosinase (MYR) (plant or gut) produces an unstable intermediate which spontaneously rearranges to thiocyanate, isothiocyanate (ITC), or to nitrile facilitated by epithiospecifier protein (ESP).

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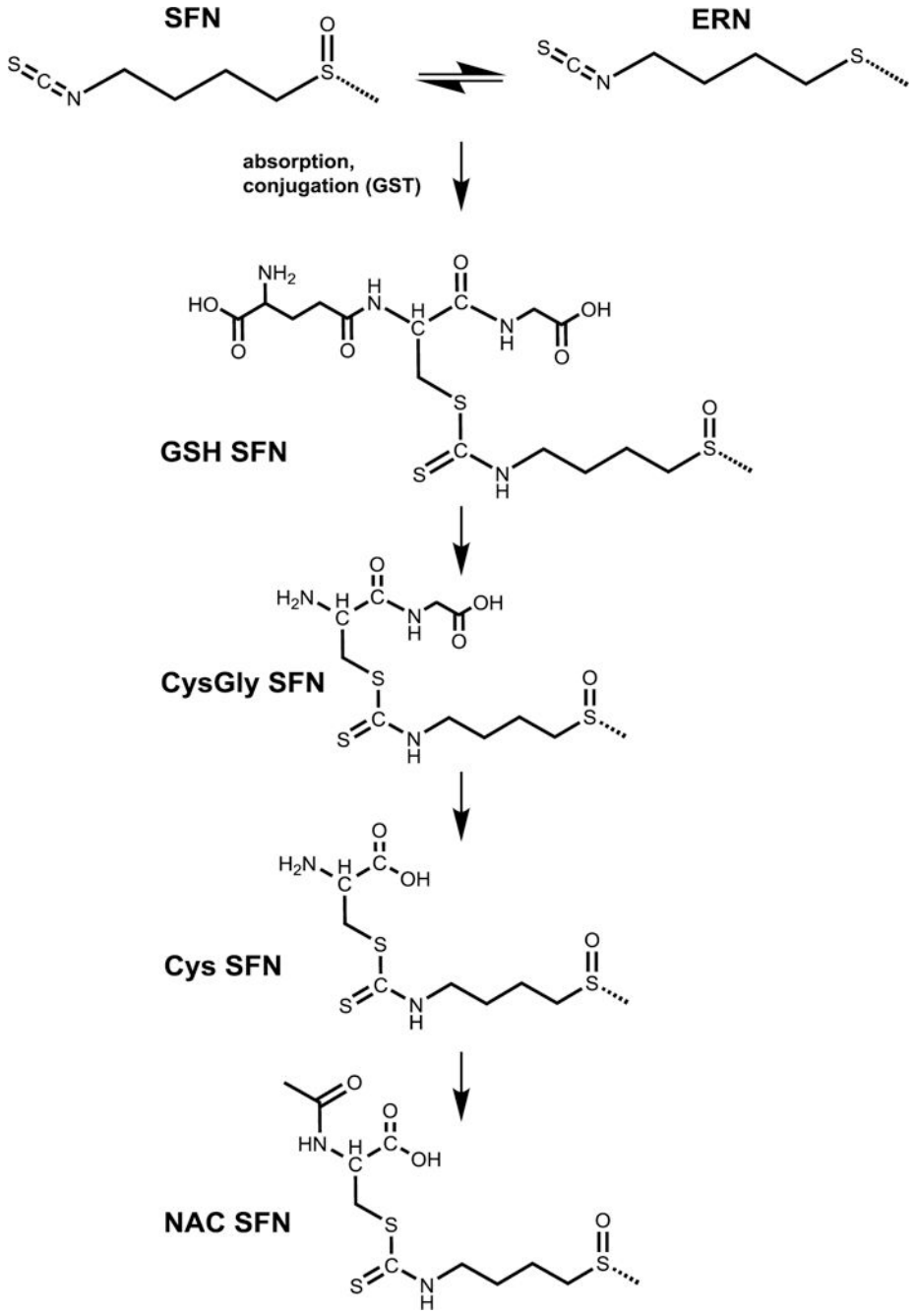

Figure 3. Mercapturic Acid Pathway.

Alkyl isothiocyanates such as sulforaphane (SFN) and erucin (ERN), produced by myrosinase (MYR) action on glucosinolates after decompartmentalization of broccoli and broccoli sprouts (chewing or processing), can be interconverted post-absorption. Either can be scavenged by glutathione (spontaneous or mediated by glutathione S-transferase (GST)) to form a glutathione (GSH) conjugate which is rapidly processed by the mercapturic acid pathway to N-acetyl cysteine isothiocyanate (ITC) which predominates in urine. SFN is used as the example here although ERN is presumably metabolized in the same manner. It is not

known whether interconversion occurs between free ITC forms, after conjugation, or from either form.

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