Current Pharmacology Reports (2018) 4:482–490
https://doi.org/10.1007/s40495-018-0163-0

CANCER CHEMOPREVENTION (R AGARWAL, KE BAYOUMY AND S YU, SECTION EDITORS)

Oxidative Stress and Bladder Cancer Carcinogenesis: Early Detection and Chemoprevention Involving Nrf2—an Integrative Approach

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Published online: 20 October 2018

Abstract
Oxidative stress is a driver of many diseases, including cancer. Nuclear factor (erythroid-derived 2) like 2 (Nrf2 or NFE2L2) provides cellular defense against oxidative stress by regulating antioxidant response element (ARE)-mediated phase II detoxifying/antioxidant enzymes. This protective role is evident from studies on bladder cancer (BCa) pathogenesis. This article reviews the impact of excessive oxidative stress on bladder carcinogenesis, aiming to understand what is happening from an epidemiological perspective concerning its burden and management, as well as to discuss the challenges including high relapse rate. Two measures are proposed for reducing the burden and better management: first, promote early diagnosis of BCa with fluorescence cystoscopy; second, increase dietary intake of nutraceuticals that demonstrate functional antioxidative Nrf2. Such an integrative approach may provide a better prognostic outcome for BCa patients or people who are at higher risk of developing BCa.

Keywords Oxidative stress · Bladder cancer · Carcinogenesis · Cancer prevention · Detection · Nrf2

Introduction

The number of new cases of invasive cancer in the USA was estimated to be 1,688,780 in 2017, which is equivalent to more than 4600 new diagnoses per day [1]. The lifetime probability of being diagnosed with invasive cancer for men is 40.8% and 37.5% for women, and it shows that early detection of cancer is important for management of cancer because the prognosis is related to the staging of the cancer. Early detected cancer of lower stage would usually have a better outcome than the same type of cancer which is in advanced or invasive stage. Specifically, bladder cancer (BCa) ranks as one of the top five cancer deaths in males over 80 years old, and cancer incidence positively correlates with age. Yet, the good news is, prevention is possible because carcinogenesis is a multistep process that also takes place over years. In general, cancer development follows three distinct yet closely interrelated phases of initiation, promotion, and progression [2, 3]. When cells are exposed to oxidative stress, DNA may go through oxidative damage [4] coupled with persisting inflammation [5] and formation of DNA adducts, resulting in cumulative genetic defects, enhanced neoplastic transformation, and ultimately, cancer formation (Fig. 1). Studies involving the well-known environmental risk factors such as cigarette smoking, exposure to arsenic in drinking water, and occupational exposure to aromatic amines and 4,4′-methylenebis(2-chloroaniline) may cause oxidative stress and show that it takes several years or even decades between exposure and the subsequent BCa formation [6]. For example, arsenic-induced BCa can be explained on the basis of "oxidative stress theory" [7]. Such an understanding of causes and pathological progression suggests BCa is preventable and cancer chemoprevention is one of the options.

Cancer research has shown some promising results [8] since the inception of "chemoprevention" by Michael Sporn in 1976 when he discussed the use of vitamin A and its synthetic analogues for preventing the malignancy of invasive epithelial cancer [9].

This article is part of the Topical Collection on *Cancer Chemoprevention*

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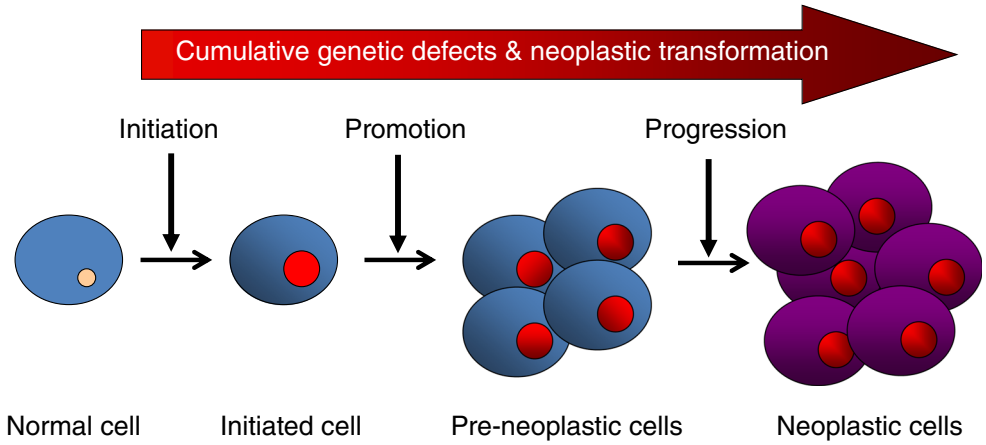

Fig. 1 Carcinogenesis is a multistep process. The initiation step is started by the transformation of the normal cell into an initiated cell that undergoes tumor promotion into pre-neoplastic cells, which later progress to neoplastic cells. Oxidative stress and inflammation, together with the accumulation of genetic defects over a lifetime of patients, will result in the formation of cancer. In reality, cancer may arise without proceeding through each of these steps. Chemopreventive agents can interfere with different steps of this process

Oxidative stress is involved in the pathogenesis of cancer. Although our understanding of how antioxidative chemopreventive agents or nutraceuticals function is incomplete, we do understand certain beneficial action results from the induction of phase II detoxifying/antioxidant enzymes [10]. These enzymes detoxify many harmful substances by converting them into hydrophilic metabolites that are readily excreted. Phase II detoxifying/antioxidant enzymes, for example, glutathione S-transferase (GST), heme oxygenase-1 (HO-1), and NAD(P)H dehydrogenase (quinone 1) (NQO1) are highly inducible in mammalian cells, animals, and humans in the presence of chemopreventive agents or nutraceuticals of diverse chemical classes [11–18]. There is a strong inverse relationship between their tissue levels and the susceptibility of tissue to chemical-induced carcinogenesis [10, 19] or ultraviolet-induced carcinogenesis [20, 21]. The coordinated induction of phase II detoxifying/antioxidant enzymes is mediated by *cis*-regulatory DNA sequences located in promoter and enhancer regions known as antioxidant response elements (AREs). The ARE transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2 or NFE2L2) plays a central role in the induction of the detoxifying/antioxidant genes [10]. Nrf2 is inhibited in the cytoplasm by the anchor protein Kelch-like ECH-associated protein-1 (Keap1). In the presence of oxidative stress or chemical inducers such as carcinogens, chemopreventive agents, or nutraceuticals, Nrf2 is released from Keap1 inhibition, then Nrf2 translocates into the nucleus, dimerizes with small Maf (sMaf) proteins, and binds to ARE consensus sequence for the production of the phase II detoxifying/antioxidant enzymes [22] (Fig. 2). Recently, the involvement of Nrf2 in epigenetic events of carcinogenesis [23] and the complexity of the Nrf2 pathway beyond the antioxidant response [24] were reported and reviewed.

In this paper, I will review the role of oxidative stress in the carcinogenesis of BCa as well as the management of BCa. Challenges that are still facing us today, preventive measures, and the role of Nrf2 will also be discussed. Hopefully, an integrative approach that encompasses early detection and chemoprevention may offer a better management. To my knowledge, this is the first review that integrates early detection of BCa with chemoprevention for the management of patients or people who are at higher risk of developing BCa (Fig. 3). The rationale behind this integration is because while chemoprevention is favorable to cancer treatment, there are challenges facing us in implementing a successful chemoprevention program in regard to what type of population should be considered and which nutraceutical for how long. There is still a gap between (1) diagnosis and staging, (2) disease progression and survival, and (3) current and potential treatments for BCa.

What Is Happening? Tough Issues and Challenges

Recently, Tang et al. investigated the impact of the intake of cruciferous vegetables in the outcome of BCa prognosis [25]. After adjusting for other prognostic factors, it was found that raw broccoli intake, among other cruciferous vegetables, had a strong and significant inverse association with BCa mortality ($\geq$ 1 vs. < 1 serving per month; hazard ratio (HR) for overall death, 0.57; 95% confidence interval (CI), 0.39–0.83; HR for disease-specific death, 0.43; 95% CI, 0.25–0.74). For a more detailed discussion of epidemiological findings from preclinical cellular and animal works involving cruciferous vegetables and their isothiocyanates (ITCs) components such as sulforaphane and phenethyl isothiocyanate, please refer to a recent review by Zhang [26]. Zhang et al. has carefully discussed primary data that support ITCs as potential nutraceuticals for clinical trials. Many cellular targets of ITCs including

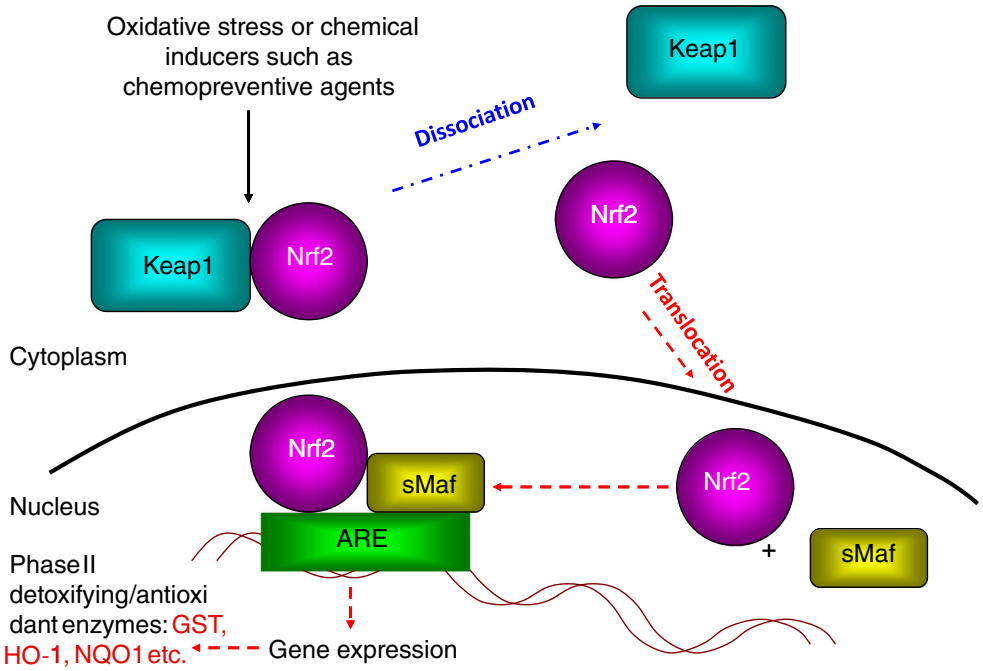

Fig. 2 A flowchart describes the major cascade of events involving Nrf2 via the Keap1-Nrf2-ARE pathway. The cytoplasmic Keap1 inhibits the translocation of Nrf2 into the nucleus. The inhibition is disrupted when there are oxidative stress or chemical inducers to dissociate Keap1 from Nrf2 binding, allowing the translocation of the transcriptional factor Nrf2 to enter into the nucleus and dimerizes with sMaf proteins and eventually Nrf2 binds to ARE to produce phase II detoxifying/antioxidant enzymes such as GST, HO-1 and NQO1

caspases, Cyclin A and B, and survivin were discussed, but our focus here is oxidative target Nrf2 in the coming sections.

In 2005, Borden LS et al. reported that the initial presentations of BCa in 70 to 80% of the cases are superficial and limited to the urothelial lining of the bladder mucosa and submucosa [27]. But this statistic did not change much after more than a decade. The most recent European Association of Urology (EAU) guidelines of 2017 still reports that there is a high risk of recurrence even for non-muscle invasive bladder cancer (NMIBC) that is confined to the mucosa or the submucosa upon diagnosis [28]. For example, among a study consisting of 252 urothelial bladder carcinomas, 67% of the tumors were low-

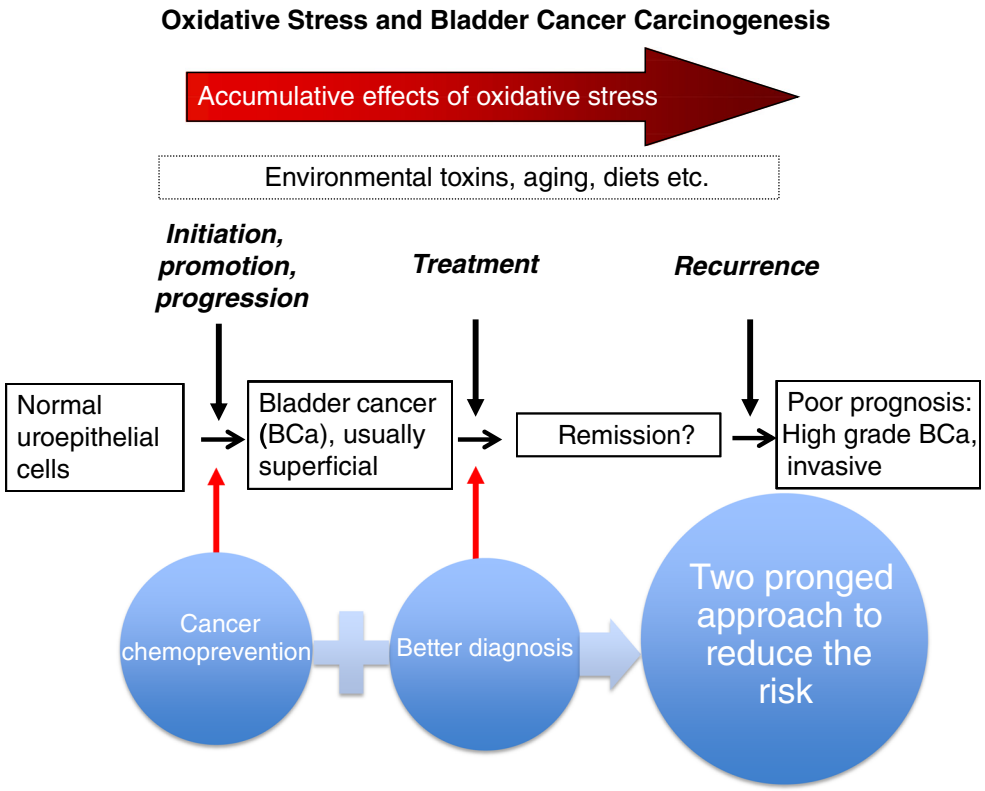

Fig. 3 The proposed integrative approach to human BCa management. This schematic presentation shows the role of oxidative stress, current understanding of clinical presentation of human BCa, and its risk of recurrence as high-grade BCa even after aggressive management. An unknown period of remission causes patients' fear and anxiety of the recurrence. The multistep process of carcinogenesis in initiation, promotion, and progression may present an opportunity to chemoprevention. To reduce the risk of recurrence and improve the survival, a better early detection can be used. To reduce the risk of BCa carcinogenesis, the cancer chemoprevention approach can be adopted

grade and 33% high-grade; 73% were superficial and 27% invasive; even after aggressive treatment such as cystectomy, radiation, instillation of Bacillus Calmette-Guérin (BCG) or mitomycin or farmorubicin or epirubicn + interferon-alpha2b (IFN), and BCG + IFN, 68% had one or more recurrence [29].

Nevertheless, superficial BCa does not mean such cancer is easier to manage. The 15 years of follow up among the group of patients with high-risk superficial BCa such as superficial transitional cell carcinoma (TCC) showed that about a third of all studied patients (29 of 86 patients = 34%) died of TCC despite aggressive local therapy such as transurethral resection alone or combined with intravesical BCG [30].

These findings suggest that at least two approaches are needed for long-term survival of BCa patients. First, a better diagnostic tool that will allow a higher specificity and sensitivity of early detection and management of superficial BCa in order to prevent its progression into muscle-invasive disease. Second, a better preventive approach that may prevent BCa formation in the first place. Thus, it makes sense to develop strategies to prevent BCa formation and also to prevent its recurrence (Fig. 3). At the early diagnostic stage, fluorescence cystoscopy will be of great importance to identify which patients are at higher risk because its detection capability had been proven superior to the conventional white light cystoscopy [31]. Visual differentiation between normal tissue and TCC is relatively easy but not so for carcinoma in situ (CIS) or nonmalignant diseases such as cystitis due to radiotherapy, chemical, or bacterial origins. They are often invisible to the naked eyes when using common white light cystoscopy [32]. Taking management of CIS as an example, the radical cystecomy is effective to achieve an excellence of tumor-specific survival rate, but nearly 40–50% of patients may have been overtreated [28, 33]. Therefore, the long-term goal of the management of this disease is minimally a hope for bladder preservation, as well as maximizing disease-specific survival.

Two-Pronged Approach

Methods of Detecting Cancer: Fluorescence Cystoscopy

The first approach can be done via the use of a photosensitizer. The EAU guideline recommends that "if equipment is available, photodynamic diagnosis (PDD) is a useful tool to target the biopsy" [28]. The American Urological Association recommends a similar management as well [34]. The basis of PDD was first reported 50 years ago when the first generation of photosensitizer hematoporphyrin derivative was used to detect various malignant tissues including skin, oropharynx, cervix, and urethra [35]. PDD is a technique that uses a shorter wavelength of light such as a blue light to activate a drug called "photosensitizer" which can convert the energy of the blue light into a form of red fluorescence at a longer wavelength. This photosensitizer shows a selective uptake or retention in human tumors thus suggesting its use for the detection of malignant lesions.

Photosensitizers 5-aminolaevulinic acid (ALA) or hexaminolaevulinic acid (HAL) are recommended by the EAU [28] but certainly, there are other candidates being developed over the years, namely hypericin [36–40] and pirarubicin [41], in order to overcome the limitations of ALA. First, ALA is a pro-drug that requires enzymatic reaction to convert itself into its active form, protoporphyrin IX, before it is able to function as a photosensitizer for its diagnostic capability. Second, 5-ALA also has a relatively lower specificity. Third, 5-ALA is easily photobleached. When protoporphyrin IX is exposed to light, several photoproducts are formed that require careful further investigation [42]. Fourth, 5-ALA exists as a charged molecule in physiological conditions, so it has difficulty in penetrating lipid bi-layers of biological membranes [43]. Overall, the reported range of sensitivity for 5-ALA is 75–100%, but very low specificity and 43–68.5% with many false positives [44]. An excellent evidence-based review of imaging techniques highlighted that the novel photosensitizer hypericin can overcome some of the limitations mentioned [45] when compared to ALA and HAL.

There are currently five reported clinical studies on BCa PDD with promising results indicating the potential superiority of hypericin over 5-ALA in some ways, but because of the differences in formulations and doses, and there are also some variations in the design of trials and samplings methods, it is best to view these trials side by side along with other existing data [36, 37, 46–48]. The past studies that performance with albumin-bound hypericin formulation has a range of sensitivity and specificity of 81–94% and 91–98.5%, respectively [36, 44, 46, 47]. The more recent ones used polyvinylpyrrolidone (PVP) to solubilize hypericin instead of albumin. It was reported that PVP-bound hypericin had a significantly higher overall sensitivity (95%) compared to the white light endoscopy (85%), and the sensitivity in detecting CIS (100%) is also higher than that with the white light endoscopy (33%); while in the diagnosis of dysplasia, the sensitivity was 85% compared with 31% for white light endoscopy [48]. The most recent study in 2015 reveals 48 out of 49 malignant lesions (98%) were detected [37], confirmed previous findings as reviewed [44], although the specificity cannot be revealed as random biopsies were not taken [37].

Measures of Oxidative Stress

Human Data

The second approach for early BCa prevention by counteracting oxidative stress is suggested by studies done in human, animal, and cell models. The focus here is on

human studies. Oxidative stress biomarker 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in DNA from leukocytes of the patients with superficial TCC of the bladder has been detected to be significantly higher than those in the age-matched healthy subjects [49]. A more recent study done among BCa patients also in the age-matched healthy subjects control fashion found that urinary levels of total sialic acid (TSA), 8-OHdG, and malondialdehyde (MDA) in BCa patients were significantly higher than the controls [50]. It was claimed to be the first report that showed an increased TSA level in spot-morning urine of BCa patients compared to the healthy controls, and TSA is suggested to be an adequate biomarker for diagnostic purposes using cutoff of 95.26 μg/g that provides the highest accuracy of 75.6% with sensitivity of 75.6% and specificity of 75.6%. Although the potential use of TSA is there, challenges remain to elucidate mechanisms that could be translated into right correlation between urinary 8-OHdG in both patients and healthy subjects while clearly distinguishing the two groups from each other. Bear in mind there is significant positive correlation of the urinary TSA and urine MDA in the healthy controls but not in the patients.

Animal Data

The relationship between oxidative status and age-related changes in urothelial cells of the urinary bladder has been investigated by using young (2 months) and aging (20 months) mice [51]. As expected, healthy young mice urothelium was found to possess a powerful antioxidant defense system that functions as a strong defense barrier against reactive species. However, in spite of the markedly elevated activities of the main antioxidants (catalase (CAT), GSH-Px, glutathione reductase (GR), and GSH), compared to the young urothelium, urothelial cells of aging bladder show significant decreased total antioxidant capacity ($p < 0.001$) and significant increased levels of lipid peroxides (MDA, about threefold) and inducible nitric oxide synthase (iNOS) as markers of oxidative stress. Moreover, lipofuscin, an aging pigment, was found in superficial urothelial cells of the urinary bladder in aging mice. Prior to this report, there was no data on testing antioxidants in the urothelium of mouse bladder.

Another study used cyclophosphamide (CP) administration intraperitoneally at the dose of 150 mg/kg to induce urotoxicity in male Sprague-Dawley rats. Mice were treated with the antioxidant melatonin to protect against CP-induced urotoxicity. Melatonin treatment (10 mg/kg) was initiated 3 days before and continued for 1 day after the CP administration. It was found that indeed, melatonin reduced CP-induced urotoxicity by these parameters: (a) reduced oxidative DNA damage, (b) reduced the bladder damage and apoptosis, (c) increased the expression of transcription factor Nrf2 as well as associated phase II enzymes NQO1 and HO-1, (d) reduced the expression of transcription factor nuclear factor-kappa-B (NF-κB), one of the key transcriptional factors in inflammatory response [52], which contributes to carcinogenesis too if activated excessively. The results of this study provides evidence that melatonin treatment favorably alters Nrf2 and NF-κB expression and, this appears to be at least in part responsible for the observed protection against CP-induced urotoxicity via melatonin's multifaceted antioxidant activities [53].

Epigenetics, Gene Expression, Role of Nrf2 and Bladder Cancer

Very recently, the epigenetic aspects of BCa have been investigated in in vitro models. Again, the findings support the hypothesis that oxidative stress promotes urothelial cell carcinogenesis through modulation of DNA methylation [54]. Though arsenic-induced oxidative stress has been studied for decades, not until recently, the effects on normal human uroepithelial cells have been reported to investigate cell signaling pathway associated with bladder carcinogenicity [55]. This is the first examination of gene expression response in primary uroepithelial cells from multiple individuals. It identified no effect levels for arsenical-induced cell signaling perturbations in normal human cells when exposed to a biologically plausible concentration range. Nonetheless, the finding is worth paying attention to because a suite of eight gene changes was consistently identified across individuals pointing to the same key signaling pathways, and oxidative stress ranks the first among others. Carcinogenicity of arsenic has been demonstrated through its ability to activate the redox-sensitive transcription factors and other signaling pathways involving NF-κB, activator protein-1, and p53 [7].

Indeed, excessive oxidative stress causes the formation of BCa. Nrf2 is the key transcriptional factor that has been shown to regulate gene expression by binding to ARE [10]. The role of Nrf2 has been investigated and confirmed by using Nrf2 knockout mice [56]. Using N-nitrosobutyl(4-hydroxybutyl)amine (BBN)-induced urinary carcinogenesis mice model, higher incidence of bladder carcinoma and invasive carcinoma in Nrf2 knockout mice have been observed. The Nrf2 activator, oltipraz has been used to reduce the incidence of bladder carcinoma by BBN in wild type but had little effects in the knockout mice. Oltipraz increased the BBN glucuronidation and decreased the urinary concentration of a proximate carcinogen of BBN and counteracted the BBN suppression of UDP-glucuronosyltransferase 1A in the bladder in the wild type mice. Since chemopreventive compounds usually work on multiple signaling pathways, using Nrf2 knockout mice is one of the key tools to ascertain whether their anti-carcinogenesis effects is Nrf2-dependent [57]. This study also demonstrates the protective role of Nrf2 in anti-carcinogenesis of BCa and other cancers.

Relevancy of Nrf2 in Human Studies

Although the polymorphism of Nrf2 and its causal effects that lead to BCa is still under investigations, there are findings supporting the association of GSTM1 null genotype with significant increased BCa risk (odds ratio (OR) 1.85, 95% CI 1.30–2.62) [58]. The impact of polymorphism GSTM1 has been assessed in a meta-analysis that supports the hypothesis that the GSTM1 null variant is a determinant of BCa susceptibility (OR = 1.41 [1.30, 1.52], $p < 0.00001$) [59].

In a study that investigated the expression of Nrf2 and Nrf2-related gene in the blood leukocytes of 51 BCa patients and 90 control males, among GSTA1, GSTP1, Nrf2, and SOD2, a significant upregulation of SOD2 expression ($p = 0.002$; 1.25-fold change) was observed in leukocytes of BCa patients. Nrf2 expression was positively correlated with GSTP1 and with SOD2 mRNA level, both in patients and controls (all $p < 0.05$). Note that there is a significant difference ($p = 0.002$) between the age of controls (58.3 ± 1.7) and patients (66.0 ± 1.3), and the distribution of non-smokers (66 in the controls and 20 in the BCa patients, $p < 0.0001$). Thus, the interpretation of these data is not that straight forward; on one hand as discussed by the authors: the positive correlation found in BCa patients and control group suggests that the constitutive expression of GSTP1 or SOD2 by Nrf2 regulation is not affected by the cancer; on the other hand they concluded that the data "suggest disturbances in SOD2 transcription in circulating blood leukocytes of males with bladder cancer" [60]. Nonetheless, this was the first study to demonstrate the interplay between constitutive expression of Nrf2 and its two target genes SOD2 or GSTP1 in human circulating blood leukocytes. It certainly contributes to a better understanding of Nrf2 in cytoprotection that deserves further clinical verification.

The cytoprotective role of Nrf2 in normal bladder cells is clearly demonstrated among the group of patients diagnosed with TCC [61]. It was found that the patients' urinary 8-OHdG was significantly higher than the controls ($p < 0.05$). The level of 8-OHdG was also correlated with the grade of TCC: grade 3 patients had a median level of twice as high as in patients with grade 2 ($p = 0.044$).

Promises and Challenges: Is Nrf2 a Friend or Foe?

The seemingly controversial role of Nrf2 in BCa formation and the chemoresistance study demonstrated the dark side of Nrf2. Nrf2 is thought to be a foe responsible for chemoresistance of cisplatin in BCa [62]. But a more careful study of the controversial role of Nrf2 in cancer was put in its rightful context by Sporn that it clearly demonstrates the protective function of Nrf2 in normal premalignant or preneoplastic tissue [57]. I have organized in this review the current findings that demonstrate a general trend of an inversed relationship between (1) either the Nrf2 or its downstream expression as antioxidant enzymatic activities or antioxidative biomarkers and (2) the status of carcinogenesis in BCa. In short, reduced antioxidant capacity in an individual has an increased likelihood of carcinogenesis (Fig. 4a). The oxidative stress-induced BCa is clearly demonstrated from the investigation that reveals changes in the oxidative status and structural alterations in the urothelial cells [51]. The most notable evidence for the development of BCa is due to increased oxidative stress seen in the Nrf2 knockout mice model [56, 57]. Most clinical findings also support the functional role of Nrf2 in preventing BCa, although some have their interpretive challenges such as the polymorphism of GSTM1 and relevant studies.

It is worthwhile to revisit the concept of chemoprevention coined by Michael Sporn in 1976 [9] whereby Sporn has said the following of the role of Nrf2 in cancer [57]:

> Overall, the question of whether NRF2 activation is 'good' or 'bad' is inadequately framed. We would

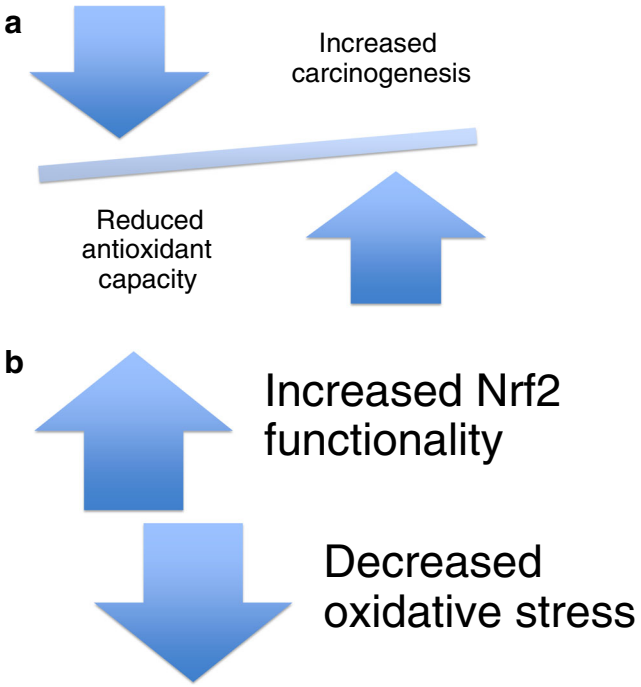

Fig. 4 Involvement of Nrf2 in BCa carcinogenesis. **a** When there is an accumulation of oxidative stress, antioxidant capacity is reduced, leading to increased carcinogenesis. **b** The critical role of redox-sensitive Nrf2 transcription factor in protecting normal bladder cells from neoplastic transformation when subject to oxidative stress is having functional Nrf2-ARE signaling. Nrf2-ARE signaling enhances the expression of phase II detoxifying/antioxidant enzymes, thus maintains oxidative stress homeostasis by producing antioxidative stress enzymes such as GST, HO-1, and NQO1 (Fig. 2). Appropriate use of chemopreventive compounds or nutraceuticals can further increase functionality of Nrf2 and reduce oxidative stress

> suggest that both sides of the argument over this apparent paradox have merit, but the answer of whether to use drugs to stimulate or inhibit the NRF2 pathway depends on context (FIG. 2). For the prevention of cancer and other chronic diseases in which oxidative and inflammatory stress contribute to the pathogenesis, enhancing NRF2 activity remains an important approach …
> In summary, there is abundant evidence that activation of NRF2 can be a safe and effective strategy for the chemoprevention of cancer and many other diseases. People have been safely ingesting NRF2 activators in their diet for millennia.

For example, ginseng is one of the "NRF2 activators" that had been safely consumed for centuries among the Chinese [63], and many phytochemicals or nutraceuticals are part of human diets since the beginning of the human race. While the "dark sides" of Nrf2 in cancer cannot be ignored, evidences show that mutations occur in Nrf2 or its inhibitor Keap1 [57]. Mutations are not normal physiology; mutations cause cancer. Cancer obtains its autonomy by hijacking the normal physiological function of cells. Under physiological condition, Nrf2-Keap1 maintains a wonderful homeostasis that keeps oxidative stress in check. It is when Nrf2 behaves abnormally that a stable overexpression that causes other problems such as chemoresistance begins. Thanks to advancement in science and technology, we now have a better understanding of both sides of the Nrf2. Sporn has rightly shared his careful thought on this issue after working in cancer chemoprevention for one of the longest times [57]. My previous review on ginseng and the role of functional Nrf2 and dysfunctional Nrf2 and his cohered [63], 2 years prior to his extensive major review [57]. For sure, there are many other pathways involved upon ingestion of nutraceuticals, Nrf2 at least plays a role in part for their health benefits.

The good and the dark sides of Nrf2 have clearly been demonstrated again in a recent study [64] 1 year after Sporn's review, confirming Sporn's conclusion. Currently, to my knowledge, the closest experiment to support Sporn is not available for BCa, but lung model. This study used Nrf2 knockout mice versus the wild type to investigate lung cancer carcinogenesis induced by urethane. It confirmed the protective role of Nrf2 in precancerous nodular examination of the lung at 4 and 8 weeks after the administration of urethane, but Nrf2 accelerated the progression in the late stage at 16 weeks [64].

While proper dosing of nutraceutical supplementations may prevent cancer formation [65, 66] (Figs. 3 and 4b) and have potential reversible epigenetic mechanisms [67], one needs to take note of the danger of overdoing it. The examples of the possible risks of over-supplementation of antioxidants should be considered by all seeking to increase antioxidant intakes [68, 69]. The context of when and how to use them should also be evaluated clinically.

Perspectives

In this review, I investigated the link between oxidative stress and BCa carcinogenesis. Though the dark sides of Nrf2 is a concern because it has been observed in many carcinomas including BCa, Nrf2 has been traditionally studied in the field of cancer chemoprevention for its protective role. Collectively, the two-pronged prevention strategy that encompasses a better detection of BCa and its chemopreventive approach, is presented here to stimulate scientific discussion and better research. Such an integrative approach may provide a better prognostic outcome for BCa patients or people who are at higher risk of developing BCa, "hopefully our patients will benefit so that he or she can say, 'I may have cancer but cancer does not have me'" [70].

Acknowledgments The author would like to thank Chung Ling Quek at Singapore Health Services (Singhealth), Singapore for reading of this manuscript. Thanks also go to Dr. Chee Loong Saw and Dr. Simran Kaur at McGill University for their helpful comments.

Funding This review is not funded by any parties.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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