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Targeting Inflammation for Bladder Cancer Chemoprevention

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Abstract

Purpose of Review Cancer of the bladder is a serious health problem with significant mortality once it progresses to advanced stages. Moreover, chronic surveillance and treatments are required to prevent the inherent high recurrence and aggressive nature of these tumors. As a result, not only the quality of life for the patients is affected but it also adds to the treatment cost, making it one of the costliest cancers to treat. Therefore, it is highly imperative to consider preventive options that have been largely neglected.

Recent Findings Although smoking prevalence is decreasing, a decline in BC incidence has not been seen, yet suggesting that smoking history and other factors still pose a threat to develop BC. With the increase of diabetic and obese populations, the risk for BC is also increasing. In spite of their modifiable nature, advancement in diagnosis, understanding of the disease, and enormous preclinical chemoprevention data, efforts in that direction for screening and primary or secondary prevention of this disease have been unsatisfactory. Moreover, no new therapeutic were approved for the last three decades; thus, the 5-year survival of BC patients has also not improved for decades.

Summary In the current review, we have discussed the central role of inflammation in the major risk factors such as smoking, diabetes, obesity, and infection leading to BC. We summarized the preclinical data of promising anti-inflammatory agents for primary, secondary, as well as tertiary prevention. Thus, we believe that developing chemopreventive strategies for bladder cancer by targeting inflammation is highly desirable.

Keywords Bladder cancer · Inflammation · Chemoprevention · NSAIDs

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Introduction

Bladder cancer (BC) is a global health problem affecting nearly 430,000 people annually, making it the 9th most common cancer worldwide. More than one third of these patients (165,000) die each year [1]. Worldwide bladder cancer incidence trends indicate its direct correlation with the level of human development [2]. Data suggest that developed countries have a threefold higher incidence of BC compared to less-developed nations (ASR of 9.5 and 3.3 per 100,000, respectively). In countries like Germany, there has been a sharp increase in BC cases among both men (35% increase) and women (75% increase) in the last three decades.

There are several identified factors like age, gender, race, and genetics that increase the risk for BC. The incidence of bladder cancer increases with age with a majority of the BC diagnoses being among 50–70-year age groups of both sexes. Since the 1950s, the incidence of bladder cancer has risen by approximately 50%. It is to be anticipated that, with the aging of the population, this trend will continue. Furthermore, BC is cancer with a very high disparity based on gender. Males are at three to four times higher risk of developing BC compared to

females. This is partly due to a higher prevalence of smoking among men compared to women. [3]. Therefore, older men with smoking habit are at very high risk for BC compared to other groups. At least 77% of BC cases are among men ranking it the 7th most common while in females, it is 19th in terms of incidence worldwide [2]. Although BC is prevalent among all ethnic groups, it is two times more frequent among whites than in the black population. Only 7% of BC cases are linked to genetic factors [4]. In general, there is a significant fraction of the population that represents a combination of all these risk factors, e.g., white, older men with a smoking habit may have very high risk for BC compared to other groups. Unfortunately, all these factors are non-modifiable; hence, they cannot have any role in prevention and thus motivates us to explore other options to achieve this unmet goal.

Other risk factors are lifestyle, occupation, or environment-related which are modifiable and can have a significant effect on the development of this disease. Some of these factors contribute positively while others are negatively associated. For example, occupations dealing with tobacco (Relative risk (RR) 1.72) or dye (RR 1.58) have a high risk for BC. The combined probability of causation was calculated to be 81.8%. Tobacco smoking remains the most imperative modifiable risk factor due to strong association between smoking history and BC. Statistically significant associations were observed for current (RR 3.14) or former (RR 1.83) cigarette, pipe (RR 1.9), or cigar (RR 2.3) smokers [5, 6] (Fig. 1). Thus, avoiding smoking or quitting alone can have a significant impact on BC in the long run. Although the prevalence rates of smoking have been declining, the absolute number of smokers has been increasing due to growing world

populations [20]. Preventing or reducing the effect of positively contributing factors or vice versa can bring down BC cancer incidence. For example, (i) reduction in occupational exposure to aromatic amines led to a decrease in BC incidence [21]; (ii) in Egypt, a marked decrease in the incidence of squamous cell carcinomas of the bladder was observed due to a reduction in Schistosoma infection [22–24]. Similarly intervening with the carcinogenesis process in the timely manner in order to prevent the initiation or progression of the disease is beneficial as well. There have been considerable advancements in our knowledge regarding the timing, sequence, and molecular changes in the carcinogenesis process of the urothelium. This provides a unique and immense opportunity for early diagnosis as well as intervention for prevention. In this review, we discuss bladder cancer prevention, including the target, cohorts, agents, and strategies.

Preventing Bladder Cancer

While BC remains a major health issue, it also continues to be a burden on the health care system due to high recurrence rates [25] and long-term monitoring of patients, resulting in the highest per patient lifetime costs among all cancers. In the USA, the annual cost of care for bladder cancer was estimated to be $US4 billion [26]. Approximately 75% of all bladder tumors are non-muscle invasive (NMIBCs) at diagnosis [27, 28]. A quarter of these tumors are T1 stage disease with an invasion of the lamina propria [29] and represent a clinical challenge because of their aggressive nature and heterogeneous outcomes [30]. In spite of sophisticated treatment methods such as combination of urine-based markers, fluorescence-guided cystoscopy, and intravesical instillations, close to 50% of these tumors recur within 2–5 years and many of which progress to muscle invasion disease that pose a high risk for metastasis in those patients [27] and death from systemic disease [31]. Therefore, regular follow-up is necessary to detect recurrences at the earliest possible stage which makes bladder cancer the most expensive of all cancer entities in healthcare systems [26, 32]. BC is preventable because, like many other cancers, it also takes significant time (~ 20 years) from the time of initiation to transform into malignant cells [33]. In relative terms, it means that most of the tumors that are diagnosed between 55 and 75 years of age were actually initiated at a young age between 35 and 55 years. Thus, screening high-risk individuals at an early stage gives an immense opportunity to intervene and prevent this disease.

Unfortunately, in spite of the highly preventable nature, in many ways, bladder cancer remains a neglected disease. Bladder cancer is diagnosed by microscopic examination of cells from urine or bladder tissue and examination of the bladder wall with a cystoscope; novel methods based on urine biomarkers or DNA are being developed and reported.
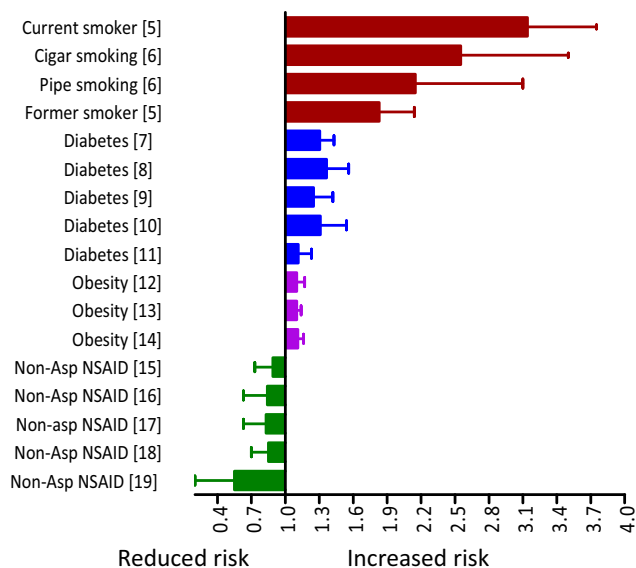
Fig. 1 Summary of relative risk between factors such as smoking, diabetes, obesity, and NSAID use. While smoking, diabetes, and obesity are associated with increased risk, regular use of NSAIDs is known to protect again BC

While these procedures are simple, there is currently no screening method recommended for people at average risk. Currently, there are neither approved preventives, nor the treatment options for BC have altered for decades. While some initial response has been noted for existing therapies, many patients experience toxic side effects and tumor recurrence despite the therapy [34–36]. Therefore, developing preventive agents against BC can prove to be pivotal.

Target Population

For any screening program, it is important to identify the high-risk target population. Since there is clear evidence on the risk association of BC with certain factors, it is easy to identify the high-risk cohorts. The risk of BC is twofold to sixfold higher for smokers than for non-smokers. As smoking is a strongly associated modifiable habit, for primary prevention, there have been many efforts to encourage people to quit smoking and as a result, the number of smokers has been going down annually. Policies to raise cigarette prices by increasing excise taxes have helped in reducing tobacco consumption. Since 2002, the number of former smokers has increased gradually compared to current smokers in the USA [37]. While this has led to a drop in the incidence of some smoking associated cancers, particularly lung cancer, bladder cancer incidence has remained unchanged for the past four decades [1]. This suggests that smoking has a long-term effect and even former smokers are still at a considerable risk for BC. So, these former smokers should be considered for screening and prevention. Recent data also indicate an increasing trend in people switching to smokeless tobacco products. While we do not have any evidence suggesting the role of these new smoking products in cancer risk, it is definitely important to be cautious and discourage their use in the primary prevention settings. Thus, smoking status should be one of the important criteria to be considered for selecting the high-risk groups.

The second criteria are health conditions such as diabetes [7–11] and obesity [12–14] since several studies have indicated their association with BC (Fig. 1). The proportion of the obese, diabetic and aging population is increasing globally and more rapidly in the developing world. Therefore, the population at risk for BC is also increasing. Since type 2 diabetes is the most common form of diabetes, affecting 85–90% of all people with the disease, this can be another criterion. Obesity itself cannot be considered a criterion for screening; there are approximately 70% adults are overweight or obese in the USA [1]. However, this, in combination with other discussed factors, can be used to shortlist the cohort group. Urinary tract infection with *Schistosoma haematobium* alone is estimated to be responsible for 50% of the BC cases in parts of Africa and Middle East countries [38, 39]. Additionally, patients with spinal cord injury (SCI) and spina bifida also experience bladder inflammation, due to bladder dysfunction, frequent bladder infections, and required catheterization, which significantly increase their risk for BC [40]. In these patients, BC tends to present itself at an earlier age and at a more advanced pathological stage than bladder cancer in the general population. More than half of SCI patients develop transitional cell carcinoma with locally advanced stage (T3 or greater) or lymph node metastases in 88% of these cases [41]. Thus, screening of these patients for early detection of bladder cancer is very important because the symptoms are atypical.

Since strong evidence is available regarding the association of bladder cancer with these cohorts, particularly population groups such as smokers (particularly men), diabetics, obese individuals (Fig. 1), or spinal cord injury patients, there is an urgent need to develop or implement screening and chemoprevention in the target population the prevention of this disease.

Risk Factors and Bladder Inflammation

Cells in the urothelium experience chronic exposure to a variety of chemicals excreted through the renal pathway. Some of these chemicals, like tobacco smoke chemicals, may have direct carcinogenic effects while others may induce inflammation of the bladder. Additionally, chronic diseases/conditions such as diabetes, interstitial cystitis (IC), bladder/kidney stones (bladder calculi) or UT infections, indwelling catheters are all known to cause inflammation of the bladder. All these factors individually or in synergy can result in the malignant transformation of bladder urothelium. Below, we have discussed the evidence for the role of these factors in inducing inflammation in bladder.

Smoking

Smoking remains a leading preventable risk factor for many diseases. Despite a large amount of evidence supporting the risks of cancer due to smoking, roughly 45 million people in the USA remain smokers [42, 43] and many more are regularly exposed to passive smoke. Ever-smokers are considered to have 2.5 times higher risk for bladder cancer than non-smokers [44]. Tobacco has been found to be responsible for about half of all bladder cancer cases [45, 46] and 40% of all bladder cancer deaths [47]. Mechanistically, smoking contributes to bladder cancer both directly and indirectly. Molecules in the tobacco smoke enter the circulation and are eliminated through the urinary pathway. The urothelium is thus exposed to a very high concentration of these toxic chemicals in the urine which may have direct genotoxic effects on it. Indirectly, the carcinogenic effects of smoking are linked to its role in

inflammation or diabetes which are risk factors for bladder cancer.

Additionally, smoking may induce systemic inflammation due to its direct effect on the inflammatory mediators. Data suggest a positive association of a higher level of inflammatory biomarkers to smoking parameters (status, burden (pack-years), and intensity (number of cigarettes per day)) [48]. Circulating levels of inflammatory molecules like platelet-activating factor (PAF) and prostaglandin E2 (PGE2) are known to be higher in smokers than in non-smokers [49–51]. Bladder endothelial cells display increased PAF accumulation and increased inflammatory cell adherence in response to cigarette smoke extract (CSE). Marentette et al. [52] concluded that exposure to cigarette smoke increases the susceptibility for developing bladder inflammation by inhibiting the activity of PAF-AH and increasing accumulation of endothelial cell PAF. Experimental evidence suggests that carbon black can promote cancer in animal models. Nanosized carbon black generated due to incomplete combustion of tobacco was found to accumulate in exposed tissue myeloid dendritic cells and macrophages. This accumulation in innate immune cells can initiate and sustain inflammation [53]. In this context of the link between smoking and inflammation, it is interesting to see that regular use of anti-inflammatory drugs was associated with a reduced risk (HR 0.87) for BC among smokers, which included both men and women. However, the risk reduction was very strong among non-smokers (HR 0.52) compared to current smokers (HR 0.80) [54].

Diabetes

Several epidemiological studies and systematic reviews have shown positive associations between diabetes mellitus and the risk for bladder cancer (up to 24% higher risk) [7–11]. Prevalence of diabetes has increased substantially over the past few decades, thus making it important to consider the role of diabetes from the prevention aspect. Diabetes is well known to cause bladder dysfunction, and recent studies have also shown a link between diabetes and bladder inflammation [55–58].

It is considered that toll-like receptor-4 (TLR4) inflammatory pathway is important for some of the biological effects of diabetes on the bladder. An increase in TLR4 expression and signaling along with increased circulating levels of specific endogenous ligands, including high-mobility group box protein 1 (HMGB1), have been observed in diabetes [55]. In STZ-induced diabetes mouse model, increased protein levels of TLR4, MyD88, and HMGB1 were observed in bladders, suggesting TLR4 pathway activation under diabetic conditions. Genetic knockdown of TLR4 or its inhibition using antagonist CLI-095 was found to be protective against diabetic complications in these animals, further supporting this idea. Further analysis showed that HMGB1, which is passively released from necrotic cells or secreted from immune cells, mediates TLR4 activation in diabetes [56]. TLR4 activation during diabetes, sustained by continuously increased levels of its ligands, leads to increased production of ROS and cytokines, which stimulate the proliferation of bladder smooth muscle cells and alter their contractile responses. Bladder cancer cells have aberrant expression of TLR4 [57, 58]; in vitro studies demonstrated that the TLR4 activation protected the cancer cells from cytotoxic T lymphocyte killing [58]. Thus, studies provide the evidence for the link between diabetes and inflammation in bladder cancer.

Obesity

Obesity is also a risk factor for many solid cancers, including bladder, and statistics indicate that there is a gradual increase in obesity rates. Meta-analysis of 15 cohort studies found that pre-obese and obese conditions had a statistically significant, 7 and 10%, increased risk of bladder cancer, respectively. The dose-response meta-analysis showed a 4.2% increase in the risk of BC for each 5 kg/m^2 increment of BMI, indicating a linear association between BMI and bladder cancer [13]. While the exact biological mechanisms driving the positive association between obesity and bladder cancer are still not well understood, it could be due to the elevated production of insulin and insulin-like growth factor-I that modify cell proliferation, apoptosis, and angiogenesis [59], thus enhancing tumor growth [60], and leading to bladder cancer [61, 62]. Obesity may also cause chronic low-grade inflammation leading to an alteration of local and systemic levels of cytokines (interleukin-6, C-reactive protein) and adipokines (leptin, adiponectin) [63], which may play a role in bladder carcinogenesis [64, 65].

Targeting Inflammation

Chronic inflammation may not only be the host's response to bladder cancer development but also actually elicit bladder carcinogenesis. While it is also important that effective cancer control requires an intact immune system, a large amount of evidence suggests that inflammation has a direct effect on cell differentiation, proliferation, and invasion [66]. Local inflammation is elicited by intravesical instillations of Bacillus-Calmette-Guerin (BCG), which prevent the invasion of tumor cells and provide long-term recurrence-free survival in patients. The clinical benefits seen in BCG patients suggest that the immune system itself plays an important role in arresting bladder cancer progression [67]. In recent years, inflammation has taken the center stage in cancer with an increasing interest and initiation of clinical trials towards immune-therapies and vaccine-based preventives.

Differential expression of key enzymes of inflammation is seen during carcinogenesis. Normal urothelial cells predominantly express high levels of cycloxygenase (COX)-1, while bladder cancer cells show COX-2 overexpression [68]. Therefore, the mechanisms that modify the expression of COX isoforms may possibly contribute to the transformation of normal urothelial cells to cancer cells. In bladder cancer cell lines, inhibition of COX activity using ibuprofen showed a reduction in cell viability via induction of proximate cell membrane glycoprotein, p75 neurotrophin receptor (p75NTR) [69]. Similarly, pharmacological inhibition of COX-2 in vivo has shown a reduction in the incidence of preneoplastic and neoplastic lesions in the BBN-pretreated bladder mucosa and reduced serum transforming growth factor-β1 and C-reactive protein (CRP) levels [70], indicating that targeting inflammation at an early stage can prevent cancer initiation. The exact mechanism through which the invasive potential of bladder cancer cells is regulated by the immune system remains unclear; however, recent evidence also points towards macrophages for their role in this process [71]. Further linking the connection of inflammation and chemoresistance and tumor recurrence found that nuclear localization of COX-2 was significantly associated with upregulation of stem cell markers Oct3/4 and CD44v6 in bladder cancer tissues [72] while inflammation mediators such as PGE2 can induce proliferation of cancer stem cells (CSCs) [73]. In another mouse study, pretreatment with celecoxib leads to a decrease in PGE2 and PGE2 induced CK14-CSC proliferation with an increase in tumor regression by cisplatin/gemcitabine [74]. These studies clearly suggest that inflammation has its role at multiple stages of bladder cancer as well as provide evidence that inhibition of inflammatory pathways using safer anti-inflammatory agents can have significant benefits by suppressing tumor-promoting mediators at various levels of carcinogenesis. The existence of indisputable evidence from a large number of preclinical and clinical studies indicates the protective role of anti-inflammatory agents [15–19]. With the recent approval of aspirin for low-risk colon adenoma patients, it is highly recommended that trials should be initiated for prevention of BC recurrence and progression.

BC Chemoprevention Using Anti-Inflammatory Agents

As discussed above, the role of inflammation in urothelial carcinogenesis is very clear and is a promising target for bladder cancer. Both human and animal model urothelial tumors are reported to show COX-2 overexpression, a key enzyme in inflammation, that was directly associated with high tumor grade and stage and is also considered as an independent predictor of disease progression and survival in humans [75].

Numerous preclinical studies using well-recognized animal models of BC support the hypothesis that suppressing inflammatory pathway may have similar preventive effects in BC [76–93] (Table 1). BBN-induced rat model of bladder cancer has been a widely used animal model for testing agents against BC in chemopreventive studies. Alternatively, we have developed a transgenic mouse model (UPII-SV40T) for chemoprevention studies in which urothelial tumorigenesis is driven by uroplakin II-driven SV40T expression [85, 90, 94, 95, 98] (Fig. 2). Studies with both COX-2 specific and non-specific agents have all demonstrated the chemopreventive efficacy of anti-inflammatory agents. These agents not only suppressed tumor growth but were also found to inhibit tumor progression, invasion, and chemoresistance. These findings provide strong support for the use of anti-inflammatory agents at all stages of cancer, starting from primary prevention to tertiary prevention. Evaluation of chemopreventive efficacy of several anti-inflammatory agents in multiple animal models providing a strong case in favor of these agents. Klein et al. [100] found that COX-2 inhibitors were able to prevent bladder tumorigenesis throughout premalignant hyperproliferation and further transformation. Likewise, celecoxib, a highly COX-2 specific agent, was observed to effectively inhibit not just development and tumor growth, but also enhanced survival in a murine model of nitrosamine-induced bladder cancer [80]. Another widely used non-steroidal anti-inflammatory drug (NSAID), naproxen, was shown to prevent bladder cancer in rat models [86, 88]. In view of the gastrointestinal (GI) toxicity and cardiovascular (CV) risk associated with certain anti-inflammatory agents, we have investigated other safer NSAIDs as chemopreventive agents against BC with similar results [85, 90]. Using a SV40T-driven muscle invasive bladder cancer mouse model, we were able to demonstrate that administration of nitric oxide-releasing naproxen (NO-naproxen), a GI safe prodrug of naproxen, at an early stage of BC was effective in suppressing bladder cancer in this transgenic mouse model [90]. Lubet et al. [88] have also reported similar results with this agent in BBN-induced rat model. Considering the CV risk associated with COX-2 specific agents, we have also explored other methods and agents for developing safer chemopreventive agents. Data suggests that targeting dual COX-LOX pathways using agents such as licofelone will protect against these side effects. Therefore, we have also tested this agent and found that oral administration of licofelone was able to suppress tumor growth as well as prevent invasion of the tumors into the muscle [85] (Fig. 2). These studies using multiple models of bladder cancer clearly indicate the potential of anti-inflammatory agents for primary and secondary prevention of bladder cancer in high risk and early stage bladder cancer patients, respectively.

Studies have also shown that these agents can have significant benefits even in the late-stage cancers in terms of tumor sensitization to standard therapies, increasing survival, etc. In

Table 1 List of synthetic agents that were tested in preclinical animal models and have shown promising chemopreventive properties against bladder cancer

Chemopreventive agent	Preclinical animal model	Reference
Anti-inflammatory agents		
Aspirin	B6D2F1 (BDF) mice/OHBBN	[76]
Atorvastatin	Male albino rat/OHBBN	[77]
Atorvastatin	Male Wistar rats/OHBBN	[79]
Atorvastatin + n-3 PUFA	Male albino rat/OHBBN	[77]
Celecoxib	B6D2F1 (BDF) mice/OHBBN	[80]
Celecoxib	Female Fischer-344 rats/OHBBN	[80]
Celecoxib	Male Wistar rats/OHBBN	[70, 81]
Celecoxib + AITC	Orthotopic rat model	[82]
Indomethacin	B6D2F1 (BDF) mice/OHBBN	[83, 84]
Ketoprofen	B6D2F1 (BDF) mice/OHBBN	[76]
Licofelone	Transgenic UPII-SV40T mice	[85]
Naproxen	Female F344 rats/OHBBN	[86–89]
Naproxen + DFMO	Female F344 rats/OHBBN	[87]
NO-naproxen	Female F344 rats/OHBBN	[86, 87]
NO-naproxen	Transgenic UPII-SV40T mice	[90]
Oltipraz	B6D2F1 (BDF) mice/OHBBN	[78]
Piroxicam	B6D2F1 (BDF) mice/OHBBN	[91]
Piroxicam + mitoxantrone	Canine model	[92]
Piroxicam + cisplatin	Canine model	[93]
Sulindac	B6D2F1 (BDF) mice/OHBBN	[76]
Sulindac	Female F344 rats/OHBBN	[87]
Sulindac + DFMO	Female F344 rats/OHBBN	[87]
Other agents		
4-HPR	B6D2F1 (BDF) mice/OHBBN	[78]
CP-31398	Transgenic UPII-SV40T mice	[94]
DFMO	B6D2F1 (BDF) mice/OHBBN	[78]
DFMO	Female F344 rats/OHBBN	[87]
DFMO	Transgenic UPII-SV40T mice	[95]
DFMO + CP31398	Transgenic UPII-SV40T mice	[95]
1,3-Diaminopropane	Male F344 rats/OHBBN	[96]
Metformin	Transgenic UPII-HaRAS mice	[97]
Rapamycin	Transgenic UPII-SV40T mice	[98]
Tamoxifen	Female mice/OHBBN	[99]

canine model of invasive BC, combining Cox inhibitor piroxicam (0.3 mg/kg) to standard therapeutic cisplatin leads to tumor size shrinkage, a favorable survival benefit compared to the group with cisplatin alone [93]. Similarly, in an orthotopic murine model of urothelial carcinoma, celecoxib was shown to improve the effectiveness of intravesical BCG, the most commonly used therapy to prevent tumor recurrence. Combining celecoxib with BCG was found to increase tumor infiltration of CD4+ T cells with a significant reduction of tumor burden in a dose-dependent manner compared with intravesical BCG alone and with untreated controls [101]. Clinical trials in humans have also confirmed similar benefits of these anti-inflammatory agents. During the 6-year follow-up in NMIBC treated with celecoxib or MMC,

celecoxib treatment led to longer disease-free survival, fewer recurrences, and adverse events [102]. While in yet another trial, celecoxib (200 mg twice daily) was able to prevent bladder cancer recurrence following TURBT, supporting a beneficial effect of celecoxib in the treatment of NMIBC [103]. CSCs or tumor initiation cells that have accumulated a set of genetic changes that can drive tumorigenesis are widely considered to be the source of tumor heterogeneity which drives drug resistance and tumor recurrence [104]. Since it is not known how many genetic events must be counteracted in order to achieve tumor eradication, preventing the evolution of heterogeneity by targeting these subpopulations may be a feasible alternative. In the orthotopic mouse model, it was found that tumor response to cisplatin plus gemcitabine was

Fig. 2 Stepwise progression of TCC in mouse model (UPII-SV40T). Data from multiple studies using well-established preclinical animal models indicate strong chemopreventive effect of several agents known to inhibit (red arrow) or modulate (green arrow) specific target proteins such as COX-2, ODC, mTOR, or TP53
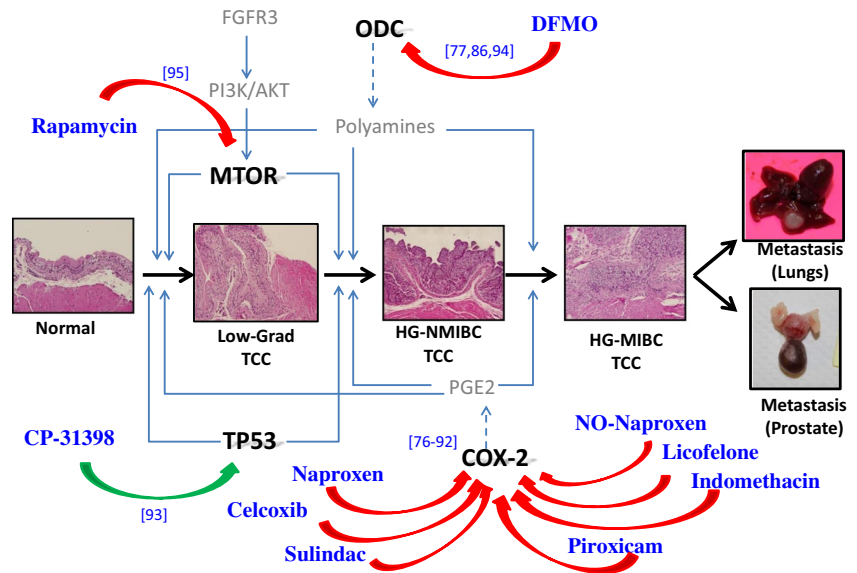
much greater when combined with celecoxib compared to the standard therapy alone. Further investigation showed that the standard therapy led to a release of pro-inflammatory molecule PGE2 that induced CSC proliferation resulting in tumor relapse and resistance to standard therapy [73]. Therefore, suppressing the PGE2 formation by including celecoxib to the standard therapy inhibited this process leading to the elimination of CSCs that drive tumor recurrence and resistance [73]. Thus, data support the use of anti-inflammatory agents

as an adjuvant to standard therapies for preventing tumor heterogeneity and drug resistance and tumor relapse that can have a significant effect on patient survival. These studies warrant further investigation of safer NSAID combination with standard therapies in preclinical and clinical settings for secondary and tertiary prevention of BC and to improve the response and benefits of currently available therapies.

Inflammation-targeting agents are being investigated in clinical trials. A randomized phase IIb/III trial for studying

Table 2 List of natural agents that were tested in preclinical animal models and have shown promising chemopreventive properties against bladder cancer

Chemopreventive agent	Preclinical animal model	Reference
1,4-Phenylene diisothiocyanate (DITC)	Male ICR mice/OHBBN	[108]
13-*cis*-Retinoic acid	Male C57BL/6 mice/OHBBN	[109]
Allyl isothiocyanate (AITC)	Orthotopic rat model	[82]
Beta-cryptoxanthin	male ICR mice/OHBBN	[110]
Bovine lactoferrin (bLF)	Male F344 rats/OHBBN	[111]
Docosahexaenoic acid (DHA)	Male Wistar rats/OHBBN	[112]
Diindolylmethane	male Wistar rats/OHBBN	[113]
Eicosapentaenoic acid (EPA)	Male Wistar rats/OHBBN	[112]
Flavokawain A	EJ cell xenograft	[114]
Flavokawain A	Transgenic UPII-SV40T mice	[115]
Flavonoids (diosmin and hesperidin)	Male ICR mice/OHBBN	[116]
Green propolis extract (WSDP)	Female Wistar rats/OHBBN	[117]
Justicidin A	TSGH8301 xenograft	[118]
Licochalcone B	MB49 xenograft	[119]
Isothiocyanates (ITCs)	UMUC3 tumor xenograft	[120]
Lupeol	Male Wistar rats/OHBBN	[113]
n-3 PUFA	Male albino rat/OHBBN	[77]
Silibinin	Male ICR mice/OHBBN	[121]
Silibinin	RT4 tumor xenograft	[122]
Silymarin	Male ICR mice/OHBBN	[121, 123]

celecoxib in preventing disease recurrence in patients who have bladder cancer has been completed recently [NCT00006124, NCT02343614] and is under investigation for MIBC [NCT02885974]. A phase 4 clinical trial is underway to evaluate the perioperative aspirin continuation vs discontinuation in BC patients [NCT02350543]. Currently, there is an ongoing randomized, phase III, multicenter, double-blind, placebo-controlled clinical trial called BOXIT (Bladder COX-2 Inhibition Trial) to determine if the addition of the oral COX-2 inhibitor, celecoxib, to standard therapy is more effective in terms of disease recurrence at 3 years compared to standard therapy alone for the treatment of NMIBC in intermediate and high-risk patients [105]. Although mounting data are in favor of the chemopreventive effect of anti-inflammatory agents against bladder cancer, it is also important to consider the side effects on renal function, particularly for the primary and secondary settings. Since it is known that glomerular function is dependent on PGE2, high doses or strong COX-2 specific agents may have negative effects on the kidneys. Interesting research is also underway to develop the renal safe anti-inflammatory agents (e.g., celecoxib-polylactic acid or celecoxib + misoprostol) [106, 107].

While anti-inflammatory agents have a strong case many other promising compounds like DFMO, metformin, etc. [77, 78, 87, 94–97, 99], and several natural agents [77, 78, 82, 108–123] (Table 2) have also been tested in preclinical animal models that need further validation in clinical settings. Many of these natural agents are not only shown to have anti-inflammatory properties but also known for other antioxidant, antiproliferative, immunmodulating properties. Several of these agents, such as diclofenac [NCT01542567], celecoxib [NCT00006124], rapamycin [NCT03298958], erlotinib, and green tea extract [NCT00088946, NCT00666562], are being investigated at various phases of clinical trials for prevention of BC. We hope that more trials will be initiated and results from ongoing trial will lead to approval of promising agents for primary and secondary prevention of BC.

Conclusion

There are several favorable reasons why BC is a preventable disease. Primary prevention can be achieved as the high-risk factors such as smoking are modifiable and other risk cohorts can be identified for screening. Secondly, the majority of bladder tumors are non-muscle invasive that are manageable when diagnosed; thus, secondary prevention can prevent the disease progression. Thirdly, like many cancers, even BC takes several years after initiation to be transformed into malignant form; thus, there is ample of time for identification, screening, and prevention. Finally, data indicates that combining preventives with standard therapies can have tumor response and survival benefits. Hence, appropriate measures can be taken to prevent recurrence and progression to an advanced stage.

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