Review

Effects of Androgen and Estrogen Receptor Signaling Pathways on Bladder Cancer Initiation and Progression

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Abstract. Epidemiologic studies have long demonstrated clear differences in incidence and progression of bladder cancer between genders suggesting that the mechanisms of development and progression in these tumors have a strong association with steroid hormonal pathways. Such observations led to preclinical studies investigating the role of androgen and estrogen receptors, as well as their cognate hormones in bladder cancer initiation and progression. Using various \textit{in vitro} cell line assays and \textit{in vivo} mouse models, studies have elucidated different mechanisms and signaling pathways through which these steroid receptors may participate in this disease. More recently, RNA expression data from multiple studies revealed a luminal subtype of bladder cancer that exhibited an estrogen receptor signaling pathway, making it a strong candidate for further consideration of targeted therapies in the future. Despite the promising preclinical data demonstrating potential roles for both antiandrogen and antiestrogen strategies targeting these pathways in different stages of bladder cancer, only two clinical trials are currently active and accruing patients for such clinical studies. Targeted therapies in bladder cancer are a large unmet need and have the potential to change treatment paradigms and improve oncological outcomes of patients with bladder cancer.

Keywords: Androgens, receptor, androgen, androgen antagonists, estrogens, receptors, estrogen, estrogen receptor modulators, urinary bladder neoplasms, carcinogenesis, disease progression

INTRODUCTION

Bladder cancer is the ninth most common malignant disease worldwide with an annual incidence of 429,800 new cases and 165,100 deaths in 2012 [1]. In the United States, bladder is the 4th most common and the 8th most lethal cancer in males [2]. The most common histological type is urothelial carcinoma and the majority (70–75\%) of patients display non-muscle-invasive bladder cancer (NMIBC) at time of diagnosis [3]. At this stage of the disease, treatment in the majority of cases involves complete endoscopic resection of the tumour followed by adjuvant intravesical chemo- or immunotherapy [4]. In contrast, cases with resistant disease or progression to muscle-invasive bladder cancer (MIBC) require radical cystectomy and systemic chemotherapy [5]. Although intravesical instillation of immunotherapy using Bacillus Calmette-Guérin (BCG) is superior to chemotherapy in prevention or at least delay of recurrence for patients with NMIBC [6], about 70\% of patients receiving BCG develop either local or
systemic side effects [7]. Furthermore, the curability after BCG instillation is limited as up to 50% of patients with carcinoma in situ (CIS) develop recurrence or progression of disease after complete response to BCG therapy [8]. Sadly, the management of bladder cancer has remained essentially unchanged, with no new efficient treatment options approved over the past few decades, and this disease therefore represents an area of great need for medical development and research.

Although sex steroid plays roles in development of the urogenital tract, and loss of ovarian steroids has been associated with urinary incontinence and bladder prolapse in postmenopausal women, the role of androgens and estrogens in the normal bladder urothelium are not well characterized. Observed epidemiological differences between males and females are obvious and suggest the potential involvement of sex steroid pathways in bladder cancer development and progression [9–12]. Indeed, the incidence of bladder cancer and its outcomes seem to be clearly influenced by gender. Men are affected by bladder cancer 3.3–4 times more often than women, even after exclusion of environmental exposures to carcinogenic chemicals and cigarette smoking [1, 2, 13–15]. Yet despite females being less frequently affected by bladder cancer, they are more likely to be diagnosed at higher disease stages (85.2% vs. 50.7% of MIBC at presentation for males) and have a greater risk of dying from their disease than men, suggesting that tumor invasion and progression may occur earlier in women [16–18]. In addition, adverse prognostic factors are also more commonly observed in females after radical cystectomy [19–21], and most studies report a higher risk of disease recurrence in comparison to males [22–24], except for one in which no difference was observed [25].

Based on the sex disparity of bladder cancer incidence and progression, many studies have been conducted to elucidate the role of sex hormones and their receptors in the natural history of bladder cancer with the ultimate goal of generating new therapeutic approaches to improve treatment outcomes. The current paper includes a non-systematic review of the literature, searching Medline for original and review articles published between 1975 and 2015 using the following keywords: androgen receptors; estrogen receptors; sex hormones and bladder cancer; gender and bladder cancer; bladder cancer progression; bladder cancer recurrence; bladder cancer outcomes. In this review article, we explore the existing evidence for involvement of sex hormone receptor signaling pathways in bladder cancer development and progression, and discuss potential new therapeutic strategies that may lead to improvements in the oncological outcomes of this disease.

**SEX STEROID RECEPTORS IN BLADDER CANCER**

**Androgen receptor (AR) expression**

Many studies have reported AR expression in human bladder cancer tissue, the effect of gender on its distribution, and the correlation of AR expression, determined by immunohistochemistry, to tumor stage and grade. There is wide variability in the percentage of AR expression detected in malignant urothelium ranging between 13% and 78% [10, 11, 26–28]. Interestingly, the number of tumors assessed in each study inversely correlated with the percentage of AR-positive tumors as 13%, 25%, 37%, 53% and 78% of tumors were found to express AR in studies considering 472, 297, 169, 49 and 9 specimens, respectively [10, 11, 26–28]. Two additional studies investigating AR expression in poorly differentiated urothelial carcinoma revealed fewer than 5% of tumors were positive [29, 30]. Other studies comparing AR expression in normal and malignant urothelium, using either control tissues from other persons [31, 32] or control specimens from a normal looking area in the same bladder [33, 34], revealed in most cases that AR expression was higher in malignant urothelium with one exception that revealed higher AR content in benign tissue [34]. No significant difference in AR distribution between males and females was detected in most studies [10, 11, 31, 32, 34–36] except in one, in which AR expression was higher in males [33]. Finally, most studies reveal an inverse relationship between AR expression and aggressive bladder cancer [10, 26, 27, 32–34], as AR expression has been predominantly detected in low-grade NMIBC (Table 1). To date, only one has study reported a significant association between AR expression and high-grade poorly differentiated tumors [31].

The available data present a variable relationship between AR expression and clinical parameters. Indeed, the largest multi-institution cohort investigating the expression of AR in 472 subjects using tissue microarrays and different antibodies indicated that AR expression was low in bladder cancer and independent of gender, grade, stage or clinical outcome [11]. The recently published mRNA expression data
Table 1
Summary of publications reporting androgen receptor and estrogen receptor expression in human bladder tissues

| Author                  | Receptor | N   | Specimen type | Stage/Grade | Correlation | Survival/Prognostic Association |
|-------------------------|----------|-----|---------------|-------------|-------------|---------------------------------|
| Boorjian et al. [10]    | AR       | 49  | tumor         | Yes – negative | NR          |                                 |
| Mir et al. [11]         | AR       | 472 | tumor         | No          | NS          |                                 |
| Williams et al. [27]    | AR       | 297 | tumor         | Yes – negative | NR          |                                 |
| Zhuang et al. [28]      | AR       | 9   | tumor         | NR          | NR          |                                 |
| Downes et al. [29]      | AR       | 13  | tumor         | NR          | NR          |                                 |
| Mohanty et al. [30]     | AR       | 16  | tumor         | NR          | NR          |                                 |
| Laor et al. [33]        | AR       | 21  | normal + tumor | Yes – negative | NR          |                                 |
| Boorjian et al. [35]    | AR       | 55  | tumor         | Yes – negative | NR          |                                 |
| Kauffman et al. [36]    | AR       | 129 | normal + tumor | NS          | NS          |                                 |
| Nam et al. [26]         | AR + ERβ | 169 | tumor         | Yes – negative (AR) | Yes – AR + ERβ |                                 |
| Mashhadi et al. [31]    | AR + ERα | 252 | normal + tumor | Yes – positive (AR) | Yes – AR      |                                 |
| Tuygun et al. [32]      | AR + ERβ | 211 | normal + tumor | Yes – negative (AR) | Yes – ERβ   |                                 |
| Miyamoto et al. [34]    | AR ERα + ERβ | 329 | normal + tumor | Yes – negative (AR + ERα) | Yes – ERβ |                                 |
| Bolenz et al. [9]       | ERα      | 198 | tumor         | NS          | NS          |                                 |
| Kauffman et al. [53]    | ERβ      | 72  | tumor         | Yes – positive | Yes         |                                 |
| Shen et al. [54]        | ERα + ERβ | 224 | tumor         | Yes – positive (ERβ) | NR          |                                 |
| Han et al. [57]         | ERβ      | 42  | tumor         | Yes – negative | Yes         |                                 |
| Kauffman et al. [58]    | ERα      | 185 | tumor         | Yes – positive | NR          |                                 |
| Kontos et al. [59]      | ERβ      | 140 | tumor         | Yes – negative | NR          |                                 |
| Croft et al. [56]       | ERβ      | 92  | tumor         | Yes – positive | NR          |                                 |
| Tan et al. [60]         | ERα + ERβ | 318 | tumor         | Yes – negative (ERβ) | Yes – ERβ |                                 |

Notes: AR: androgen receptor; ER: estrogen receptor; N: number of samples; NR: not reported; NS: not significant.

Supporting sub-classification of bladder cancer also did not observe a marked presence of AR or evident expression of its related pathways as key markers driving this tumor sub-classification [37–39]. Thus, while the data taken together makes a strong case for AR involvement in bladder cancer, there clearly is a need for further large studies to better ascertain the role of AR, if any, in the gender differences in this disease.

An important factor to consider when studying the activation of AR, as well as ERs, is the recruitment of transcriptional modulating factors (coactivator and corepressor proteins) which interact with ligand-bound receptor dimers, and are critical mediators of processes necessary for gene expression such as chromatin remodeling. Thus, altered expression of these positive and negative coregulators can result in significant changes in steroid receptor-mediated cell processes, and be associated with development and proliferation of tumors [35]. There are well over 200 coregulators known to interact with AR and ER pathways and of those, only a very few have been initially explored in the context of bladder cancer development [35, 40]. Notably, members of the steroid receptor coactivator family are expressed in human bladder tumors, and knockdown of their expression in human bladder cancer cell lines reduces cellular proliferation [35]. Moreover, the AIB1/NCOA3 coactivator has been associated with poor patient prognosis [41] and proliferation mediated by Akt and E2F1 pathways [42]. Nonetheless, the study of the expression and function of coregulators in bladder cancer is still in its infancy and there is a significant need for research in this field.

Roles of androgens and AR in bladder cancer:

In vitro studies

Several in vitro studies suggested significant biological effects of androgens, AR and their signaling pathways on bladder tumor growth and progression. Using a dye transfer method in 1990, Kihara et al. reported a reversible inhibitory effect of testosterone on gap junctional intercellular communication for JTC-30 and JTC-32 human transitional cell carcinoma cell lines [43]. In another study using small interfering RNA (siRNA) directed against AR, cell proliferation, apoptosis, migration capacity, growth and metastasis-related gene expression were all affected by AR knockdown [44]. After silencing AR, tumor cell proliferation was inhibited and migration capacity was decreased while apoptosis was increased. Additionally, expression of the evaluated growth-related genes, cyclin D1 and Bcl-xL, and the metastasis-related gene MMP-9 were decreased by AR siRNA knockdown, supporting a role for AR signaling in these biological processes [44].
Complementary data obtained by Hsu and colleagues demonstrated that AR transfected cells form more colonies in soft agar than do non-transfected controls in a neoplastic transformation assay induced by exposure to the carcinogen 3-methylcholanthrene (MCA). They also concluded that urothelial AR could modulate p53 signaling and DNA damage repair, and in turn bladder tumorigenesis, after observing that AR knockdown did not impact tumorigenesis in neoplastic transformation assays conducted in the presence of a p53 inhibitor. These in vitro findings were corroborated by in vivo experiments, suggesting that AR might promote cancer transformation in normal urothelium through modulation of p53 tumor suppressor functions [45].

Further exploratory studies have investigated the impact of androgens and AR signaling on the epidermal growth factor receptor (EGFR)/ERBB2 pathway, as well as the effects of androgens on β-catenin signals associated with AR expression in diverse human urothelial carcinoma cell lines, including UMUC3, TCC-SUP, 5637 and J82, as well as 293T human embryonic kidney cells transfected with AR [46, 47]. Collectively, these studies offer additional support to the hypothesis that AR contributes to bladder cancer progression. They also provide mechanistic insight into the pathways (EGFR/ERBB2 and androgen-induced β-catenin/TCF/LEF1 activity) through which AR may achieve this effect, and suggest androgen deprivation as a potential therapeutic approach in bladder cancer. Additional evidence for role(s) of androgens and AR in bladder cancer progression includes the observation that androgen deprivation and/or treatment with the antiandrogen flutamide, as well as AR knockdown was able to suppress cell proliferation assessed in vitro assays [48].

Roles of androgens and AR in bladder cancer:
In vivo studies

Many years ago, the effect of testosterone on bladder carcinogenesis in female Wistar rats was investigated. Supplementation of testosterone increased the incidence of bladder tumors in intact animals but not in oophorectomized rats, suggesting that the combined action of testosterone and estradiol in bladder favoured the formation of tumors [49]. Other investigators also studied the development of chemically-induced bladder cancer using N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in both intact and gonadectomised, male and female Wistar rats, receiving diethylstilbestrol (DES) and testosterone supplementation. They observed that DES reduced the incidence of bladder tumors significantly in male rats, while in the female animals the incidence was higher in spayed animals supplemented with testosterone than in the intact females. From this, the authors concluded that DES inhibited bladders tumors in male rats while testosterone stimulated tumor development in female rats [50].

More recent in vivo studies have further supported the critical role of AR and androgens in bladder carcinogenesis in mice. Miyamoto and colleagues observed that none of their AR knockout (ARKO) male or female mice developed BBN-induced bladder cancer, whereas 92% of wild-type males and 42% of wild-type female mice did [48]. Castration reduced BBN-induced bladder cancer to 50%, consistent with a pro-tumorigenic role for androgens. Surprisingly, 25% of BBN-treated ARKO mice supplemented with DHT developed bladder cancer suggesting that androgens and/or their metabolites may be involved in bladder carcinogenesis through AR-independent mechanisms. Thus, the authors concluded that both androgens (via AR and non-AR pathways) and AR (via androgen-dependent and androgen-independent signals) contribute to bladder cancer formation. Nonetheless, when analysing cancer progression, they found a correlation with AR-expressing tumors having higher cell proliferation and lower apoptotic rates in comparison to AR-negative tumors [48].

In another study analysing mice lacking only urothelial AR, the incidence of BBN-induced bladder cancer was lower and survival rate was higher than in wild-type littermates. Urothelial AR facilitated transformation of normal cells to carcinoma, likely via p53-PCNA DNA repair signaling [45]. Another possible mechanism involves the UDP-glucuronosyltransferases (UGTs), which are important phase II drug metabolism enzymes that are known to protect epithelia by detoxifying potential carcinogens by catalysing glucuronidation. Members of the UGT1A superfamily have been implicated in the metabolism of aromatic amines, and thus are considered key enzymes involved in detoxification of major bladder carcinogens [51, 52]. The AR appears to reduce the expression of UGT1A in the bladder. For instance, ARKO mice display higher expression of Ugt1A than their wild-type counterparts, and castration of wild-type mice and subsequent DHT supplementation was associated with up-regulation and down-regulation of Ugt1A, respectively [51].
Together these results suggest that AR and androgens may utilize several distinct mechanistic pathways to promote bladder tumorigenesis.

**Estrogen Receptor (ER) expression in bladder cancer**

Expression of ERα and ERβ in bladder cancer was reported in some of the AR studies mentioned above [26, 31, 32, 34] as well as in independent studies (Table 1) [9, 53–61]. The reports on ERα expression in different studies have been inconsistent. While some have found ERα to be expressed at significantly higher levels in immortalized urothelial and bladder cancer cells than in benign urothelial cells [55], others noted a markedly lower expression of ERα in human bladder cancer tissue and various cell lines [34, 53, 54, 56, 60]. While disparities in cell line expression can be explained by multiple factors, the expression of ERα and ERβ in human tissues have been more uniformly reported across most studies. Only one study has reported lower expression levels of ERα and ERβ in tumor tissues in comparison to benign bladders, with the loss of ERα expression strongly associated with higher stage and grade tumors [34]. The majority of studies observed low to undetectable ERα expression with increased ERβ expression rates found in almost all human bladder cancer tissues. The ERβ expression rates reported for human tissue varies widely between 2.5% and 81% depending on several intrinsic and technical aspects [31, 34, 54, 57–59, 61]. Some of the intrinsic aspects are related to utilization of different tissue areas (e.g. trigone versus other areas of the bladder wall), different grade and stages of disease, and history of previous treatment, while technical aspects include those typically inherent to immunohistochemistry methods such as differences in type and concentration of the antibodies utilized, differences in collection and fixation protocols, and different positivity criteria. Despite the wide variance in the ERβ expression rates, in most studies, a positive ERβ expression has been associated with higher stages and grade of urothelial carcinomas [34, 53, 54, 56–58, 60], with some studies also showing a correlation of receptor expression levels with oncological outcomes [34, 53, 56, 57, 60]. These data are in line in most of the studies supporting the emerging consensus that ERβ is the dominant ER present in bladder epithelium and cancer [9, 32, 34, 53, 54, 56–58, 60].

Recently, RNA profiling data have demonstrated the existence of multiple molecular subtypes of bladder cancer, and somewhat surprisingly revealed an estrogen-like signaling signature in a subset of tumors. Choi and colleagues reported the basal, luminal, and p53-like subgroups of MIBC in resemblance to human breast cancers [38]. In particular, the luminal subtype displayed an ER/TRIM24 gene expression pathway [38]. In an independent study yielding complementary results, other investigators identified two main human high-grade bladder cancer subtypes, referring to them as luminal-like and basal-like tumors, because of the similarities of the RNA expression profiles to human breast cancer types [39]. These authors also observed a marked presence of GATA3 (a strong epithelial marker for primary breast cancer and also seen highly expressed in bladder) and ER pathway signaling in the luminal subtype [39]. Finally, data from The Cancer Genome Atlas also yielded similar findings from their mRNA, miRNA and protein analyses, which led to classification of bladder tumors into 4 clusters, with clusters I and II exhibiting papillary histology and an elevated ER signaling signature with marked similarity to luminal breast cancer [37]. The identification of ER signaling pathways in only a subset of tumors analyzed in these mRNA expression studies may be an important contributing factor to the variable results previously observed by immunohistochemistry studies. This may be an important consideration for future study design.

**Role of estrogens and ERs in bladder cancer:**

**In vitro and in vivo studies**

Several groups have demonstrated a proliferative response of bladder cells to 17β-estradiol (E2) stimulation. When studying normal human bladder urothelial cells, immortalized urothelial (E6, E7, and UROtsa) and bladder cancer cell lines (HTB-9 and T24), Teng et al. observed that both ERα and ERβ contribute to estrogen-induced G1/S phase progression and proliferation in urothelial cells when stimulated with E2, and other ERα and ERβ selective agonists, propyl pyrazole triol (PPT) and diarylpropionitrile (DPN), respectively [55]. An increased expression of ERα was noted in the bladder cancer cell lines and immortalized urothelial cells in comparison with benign urothelium cells, along with induced expression of cyclin D1 and cyclin E, possibly resulting in dysregulated cell proliferation [55]. Others have reported on the ability of E2 and the antiestrogens 4-hydroxytamoxifen (4HT), raloxifene, andICI 182,780 to affect growth of RT4 and 5637 cell lines which originated from a superficial and an invasive bladder cancer, respectively. The inhibitory effect of raloxifene was reflected by the combination of
increased cell apoptosis and inhibition of cell proliferation via alteration of cell cycle regulatory genes [54, 62, 63]. Both mechanisms were suggested to be mediated by both ERα and ERβ [62].

Different mechanisms have been proposed to explain the effect of estrogens and the potential role of ERα and/or ERβ in the development and progression of bladder cancer. The enzyme, UGT1A is differentially regulated by estrogens in normal versus neoplastic urothelium, and it has been suggested that it plays a protective role in both development and progression of bladder cancer [64]. The expression of UGT1A was studied in a tissue microarray built using 145 human bladder tumor tissues and 101 benign appearing tissues from bladders of patients with tumors. The expression levels, measured by immunohistochemistry, were higher in the matched benign tissue and non-muscle-invasive tumors than in cancer tissues and muscle-invasive carcinomas, respectively. A significant association was observed between the lower expression of UGT1A and high-grade non-muscle-invasive tumor progression as well as disease-specific mortality in subjects with muscle-invasive tumors. UGT1A levels were increased in normal urothelium and decreased in tumor tissues after stimulation with estradiol, and this effect was reversed by antiestrogens. Similarly, ovariectomy in mice was accompanied by a downregulation of Ugt1a expression [64]. Taken together with the AR data discussed previously, this data suggests that both androgens/AR and estrogens/ER signals participate in the modulation of UGT1A expression in the bladder, and therefore may play an important role in the ability of the urothelium to detoxify carcinogens and prevent bladder tumorigenesis. The protective effect of estrogens mentioned above are in line with data showing the down regulation of UGT1A promoted by androgens, consistent with the male dominance in the bladder cancer incidence. Although these investigators were not able to show gender or age differences between the ER and UGT1A expression in bladder tissues, there was strong indication that modulation of UGT1A could potentially be an underlying mechanism to explain gender differences in this disease.

To specifically study the effect of ERβ on bladder cancer, investigators used 4-[2-(phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl)]phenol (PHTPP), a selective ERβ antagonist to treat bladder cancer cells and obtained similar effects that those produced by in vivo experiments utilizing ERβ knockout mice in carcinogen-induced (BBN) bladder cancer model [65]. Knock-out of ERβ reduced bladder cancer incidence in female mice to 23% in comparison to 75% observed for the wild-type controls. Mechanistic studies found that specifically targeting ERβ suppressed the expression of minichromosome maintenance complex component 5 (MCM5) and bladder cancer cell growth. MCM5 is a DNA replication licensing factor member of the MCM2-7 complex and essential in mediating DNA replication initiation and elongation in eukaryotic cells, also involved in tumor cell growth. These experiments demonstrate a mechanism by which ERβ, via MCM5 expression, can control bladder cancer growth and invasion. These observations provide evidence that strategies targeting ERβ (and downstream regulators such as MCM5 regulation) can effectively control bladder cancer in carcinogen-induced models and potentially play important therapeutic role in this disease [65].

The data supporting a role for ERα in bladder cancer contrasts with that for ERβ, as the former may exert both pro- and anti-tumorigenic effects. For instance, a recent study studying cancer associated fibroblasts (CAF) revealed that stroma in tumors had higher expression of ERα and suggested that the receptor could promote bladder cancer invasion via modulation of chemokine (C-C motif) ligand 1 (CCL1) and interleukin-6 (IL6) signaling [66]. Conversely, other data from the same laboratory, in which carcinogen-induced malignant transformation was reduced in urothelial cells expressing ERα in comparison to those that were ERα negative, supports the concept that ERα plays a protective role in bladder [67]. The induction of inositol polyphosphate-4-phosphatase, type II (INPP4B) in the ERα-positive cells suggests a role for reduced AKT activity and consequently cell growth in this protective effect [67]. Correlative studies using in vivo ERα knockout mouse models provided further support for an ERα protective role in cancer initiation and growth, at least in part through modulating the activity of procarcinogenic pathways, such as the INPP4B/AKT pathway [67]. These authors were able to show through quantitative data that INPP4B mRNA was induced by ERα in bladder cancer cells (UMUC3 and T24) and also non-malignant cells (SVHUC), and that INPP4B protein levels were also increased in cells with ERα. They further explored the postulated regulation of INPP4B expression at the transcriptional level, by showing that ERα can upregulate INPP4B promoter activity in UMUC3 and HEK293 cells. Confirmatory data was obtained from chromatin immunoprecipitation (ChiP) assays in ERα-transfected T24 cells
showing that ERα can bind to the –2520 to –2287 region of the INPP4B promoter which encompasses a non-classical estrogen response element [67]. Finally, ERα overexpression increased levels of p27 suggesting that this cell cycle inhibitor along with the inhibition of AKT, possibly through induction of INPP4B, contributes to the receptor-dependent reduced bladder cancer cell growth [67].

Endocrine based therapeutic trials

Preclinical AR and ER studies

Antiandrogen-based studies have shown promising preclinical data supporting a role for these AR inhibitors in modulating or inhibiting bladder cancer growth. In an UPII-SV40T transgenic mouse model, investigators observed that castration reduced the volume of bladder tumors in comparison with intact wild male mice and castrate male mice treated with DHT [68]. The UPII-SV40T is a transgenic mouse model utilizing a chimeric gene that uses the uroplakin II gene promoter to express the oncogene, simian vacuolating virus 40 large T antigen in the urothelium; animals bearing low copy numbers of the SV40T transgene reliably develop CIS while those with high copy number develop invasive and metastatic tumors [69]. This tumor model does not recapitulate the role of carcinogens in tumorigenesis, but provides a good platform to study interventions addressing specific genetic alterations. Observing that high-grade tumors could be associated with low AR expression and also that steroid hormone receptors other than AR may be activated by dihydrotestosterone, lead these researchers to study this issue in bladder cancer cell lines and UPII-SV40T urothelial explants expressing little or no AR [68]. They observed that DHT was still able to increase proliferation despite the apparent absence of AR. Thus these authors concluded that in addition to an active androgen-mediated classical AR signaling pathway, there appears to be an alternative AR-independent pathway promoting bladder cancer cell growth as well [68], reminiscent of the conclusion reached in studies of BBN-induced carcinogenesis for the ARKO mice noted above [48].

In contrast to the pro-tumorigenic role of androgens such as DHT, antiandrogens have been shown to inhibit tumorigenesis as assessed in the BBN carcinogen-induced tumor model [45]. Treatment of mice with ASC-J9, an AR degradation enhancer ASC-J9 that reduces AR expression suppressed bladder tumorigenesis such that there was a 20% incidence in treated mice versus 80% in the control animals treated with BBN alone [45]. In addition to its use as a monotherapy, several antiandrogens have been tested in combination with standard or new intravesical immunotherapies. Investigators studying the new compound protein aggregate, magnesium-ammonium phospholinolate-palmitoleate anhydride (P-MAPA), as an intravesical immunotherapeutic agent in addition to chemotherapy to address the AR in a female rat model with NMIBC observed that the addition of hydroxyflutamide to either P-MAPA or BCG immunotherapy resulted in better response of the bladder tumors than to either of immunotherapies alone, purportedly by enhancing the interferon signaling pathway [70]. Another study also reported enhanced BCG efficacy in limiting bladder cancer progression utilizing a combined therapy approach in which either ASC-J9 or hydroxyflutamide, another AR antagonist, was added to BCG in female rats with BBN-induced bladder cancer [71]. The mechanism of action seems to be related to modulation of key immunologic factors, including integrin-α5β1, TNFα, and interleukins, specifically IL-6, which are up-regulated by ASC-J9 and are thought to enhance the efficacy of BCG on bladder cancer cells [71].

Tamoxifen is an antiestrogen that exerts its effect, in part, by competitively blocking estradiol from binding to the ER. An important characteristic of tamoxifen is that its direct effect on ER action is different in various tissues, allowing it to selectively inhibit or stimulate estrogen-regulated functions in various tissues or organs. For this reason it is also classified as a selective estrogen receptor modulator (SERM). In an early preclinical study, tamoxifen was employed as a chemosensitizing agent that increased the response of bladder cancer cell lines to the cytotoxic agents, methotrexate, vinblastine, doxorubicin, and cisplatin in a concentration dependent manner. The basis for this effect was not established, but appeared to be through a mechanism other than modulation of multi-drug resistance gene (MDR-1) as its expression was not affected [72]. This early study did not discuss tamoxifen’s antiestrogen effect, but indicated that tamoxifen had a direct cytotoxic effect on bladder cancer cells, leading to the conclusion that intravesical application of this drug should be further explored [73].

Thereafter, Sonpavde and colleagues demonstrated that tamoxifen and raloxifene, both ER antagonists of the SERM class, were able to suppress growth of 5637 transitional cell carcinoma xenografts in nude mice. The average tumor volumes decreased in all studies utilizing the SERMs and 17 out of 30 treated mice
had no detectable tumor [63]. When assessed in a chemoprevention setting with the BBN-induced bladder cancer model, tamoxifen was able to reduce tumor incidence from 76% in the control group (BBN alone) to 10–14%, depending upon whether tamoxifen treatment was continued after concurrent tamoxifen and carcinogen treatment [74].

Clinical AR and ER studies

In a large Japanese multi-centre retrospective cohort study of patients with prostate cancer, investigators assessed the impact of androgen deprivation therapy (ADT) in those who also developed primary bladder cancer. They identified 239 men out of 20,328 subjects diagnosed with prostate cancer between 1991 and 2013. Patients who received ADT for their prostate cancer had a significantly lower recurrence rate (40% versus 76%, \( p < 0.001 \)) for bladder cancer, and also fewer recurrence episodes (5-year cumulative recurrence: 0.44 versus 1.54, \( p < 0.001 \)), when compared to patients who did not receive ADT [75], suggesting a possible therapeutical role for ADT in this disease. Two reports discussed the potential therapeutic role of tamoxifen in advanced bladder cancer disease. One early case report suggested that the regression of metastatic urothelial carcinoma disease could have occurred because of the initiation of tamoxifen indicated for gynecomastia [76]. In another study assessing the potential chemosensitizing effect of tamoxifen, a cohort of 30 patients with bladder cancer (9 with muscle-invasive disease, 21 with unresectable or metastatic disease), was treated with high-dose tamoxifen in addition to systemic chemotherapy including cisplatin, methotrexate, and vinblastine (CMV). The lack of control group preclude major conclusions, but the overall response rate of 58% (1 complete and 14 partial responses) was comparable to historical conventional cisplatin-based combinations [77].

Limitations and clinical implications

As evidenced in this review, there are very few clinical studies targeting AR and ER in bladder cancer despite the strong and growing body of preclinical data supporting their association in both carcinogenesis and tumor progression via various proposed mechanisms. An acknowledged limitation of the preclinical data is the few number of cell lines that are utilized in the \textit{in vitro} experiments, and how these findings are correlated and reproduced in human tissue. Similarly, the relevance of bladder cancer mouse models in humans may not yet be completely known.

From the investigational and mechanistic stand point, this work has been instrumental to increase our understanding of the potential roles that these nuclear steroid receptors have in the development and progression of bladder cancer, but more translational and clinical work is required to turn these laboratorial discoveries and observations into viable clinical management tools for patients with this disease. For instance, clinical trials testing the hypothesis of adding ADT or SERMs to current management strategies to different stages of the disease are needed. There are only two clinical trials currently registered in the ClinicalTrials.gov addressing this topic. One is investigating an antiandrogen drug (NCT02605863), and the other is studying a SERM (NCT02197897), both in NMIBC. Targeting both androgen and estrogen receptors and their related signaling pathways has the potential to improve current prevention and treatment strategies in bladder cancer, impacting significantly these patients’ oncological outcomes.

CONFLICTS OF INTEREST

There are no conflicts of interest to report.

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