The emerging role of the androgen receptor in bladder cancer

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Abstract

Men are three to four times more likely to get bladder cancer than women. The gender disparity characterizing bladder cancer diagnoses has been investigated. One hypothesis is that androgen receptor (AR) signaling is involved in the etiology and progression of this disease. Although bladder cancer is not typically described as an endocrine-related malignancy, it has become increasingly clear that AR signaling plays a role in bladder tumors. This review summarizes current findings regarding the role of the AR in bladder cancer. We discuss work demonstrating AR expression in bladder cancer and its role in promoting formation and progression of tumors. Additionally, we discuss the therapeutic potential of targeting the AR in this disease.

Key Words

- ▶ bladder cancer
- ▶ androgen receptor
- ▶ endocrine therapy

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Introduction

Bladder cancer in the US is estimated to account for ~74 000 new cases of cancer and ~16 000 deaths in 2015 (Siegel *et al.* 2012). Overall, >90% of bladder cancer cases are urothelial cell carcinomas which are malignancies of the specialized transitional epithelium lining the organ (Crallan *et al.* 2006). Urothelial cell carcinomas of the bladder (UCCB) are classified into two categories; non-muscle invasive and muscle invasive (Knowles & Hurst 2015). Though non-muscle invasive disease is more prevalent and very treatable, tumor recurrence is common which can lead to invasion (Carneiro *et al.* 2015). Muscle invasive bladder cancer is far more problematic with a higher likelihood of metastasis that ultimately leads to death (Knowles & Hurst 2015). Treatment options for metastatic bladder cancer include chemotherapy regimens such as methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) and gemcitabine and cisplatin (GC) (Kim 2012). However, despite positive responses in some individuals, patients succumb to the disease due to the

development of resistance (Wen *et al.* 2015). Research is ongoing to better understand the molecular mechanisms of this disease so that novel treatments can be identified (Carneiro *et al.* 2015). Despite these efforts, treatment options have remained largely unchanged for the past 30 years thus demonstrating the need for further studies on the factors driving this malignancy (Powles *et al.* 2014).

Further complicating our understanding of bladder cancer is the gender disparity that characterizes its diagnosis. Men are three to four times more likely to develop bladder cancer than women (Siegel *et al.* 2012). Reasons proposed for this disparity have been thought to include environmental as well as lifestyle factors such as smoking (Hartge *et al.* 1990). However, further investigation that controlled for these variables still showed that there is a higher likelihood for men to develop bladder cancer than women (Hartge *et al.* 1990). Although bladder cancer is not traditionally thought of as an endocrine-related cancer, the theory that the androgen receptor (AR)

and androgen signaling is involved in the etiology and progression of this disease has been proposed.

The AR is a steroid hormone receptor with a size of 110 kD responsible for mediating androgen (i.e., testosterone, dihydrotestosterone) signaling (Fig. 1) (Proverbs-Singh *et al.* 2015). The AR functions as a transcription factor. When bound to androgen, the AR is released from heat shock proteins (HSP), becomes phosphorylated, forms a homodimer, and translocates from the cytoplasm to the nucleus. In the nucleus, the AR binds to androgen response elements in the genome, and along with co-regulators, controls gene transcription (Mudryj & Tepper 2013). Alternative mechanisms which activate the AR in the absence of androgens also exist. Signals initiated by other receptors such as epidermal growth factor

receptor (EGFR) can lead to activation of the AR independent of androgen (Lamont & Tindall 2011). Additionally, low molecular weight AR isoforms missing the ligand binding domain can signal without androgen (Devlin & Mudryj 2009).

Androgen signaling has been linked to regulation of proliferation, motility, and cell death (Culig & Santer 2012). The AR has been shown to play a role in malignancies of the prostate, kidney, lung, breast, and liver (Chang *et al.* 2014). The AR's role in oncology is best described in prostate cancer where it plays a critical role in all stages of tumorigenesis. Recent studies suggest that there is a higher than expected association between prostate cancer and bladder cancer (Marcinkiewicz *et al.* 2012). This suggests that AR signaling may be one

Figure 1

The basic mechanisms of AR signaling. (1) The canonical mechanism, where testosterone (T) interaction with the heat shock protein (HSP)-bound AR results in an AR confirmation change, dissociation from the HSP, phosphorylation, dimerization, and translocation into the nucleus, where the AR, in concert with co-regulators promotes gene transcription. (2) In an alternative mechanism, AR co-regulators, including kinases, interact with

the AR to modify its confirmation driving its dissociation from HSPs, translocation into the nucleus, interaction with co-regulators, and promotion of gene transcription. (3) In a mechanism described in prostate cancer, a truncated AR molecule that is missing the ligand binding domain can translocate into the nucleus in the absence of ligand and along with co-regulators promote gene expression.

mechanism that contributes to bladder cancer development. To explore the possible role of AR in bladder cancer, several labs have defined AR expression patterns and function in this disease. These studies argue that the AR is involved in the etiology and progression of bladder cancer. However, a complete understanding of the AR in bladder cancer and what this information means clinically is still needed.

In this review, we will provide an overview of the landscape pertaining to the AR's role in bladder cancer. First, research showing AR function in normal bladder tissue will be presented to demonstrate its role and function in this organ. We will then present work highlighting AR expression and function in experimental models and in human bladder tumors. Additionally, we will review the molecular mechanisms by which the AR may promote bladder cancer and, finally, we will discuss the therapeutic implications of AR's role in bladder tumorigenesis.

Role of the AR in urothelial development and normal bladder function

Studies on the development of the internal male genital organs found that the prostate, bladder, membranous parts of the urethra, and bulbourethral gland are all derived from the endoderm of the urogenital sinus (Mizuno 1994, Thomas *et al.* 2008). The development of the prostate is dependent on the presence of androgens whose action is mediated by the AR. Therefore the urogenital sinus expresses the AR (Mizuno 1994). Studies on the expression of AR in adult tissues were initiated following the cloning of the AR and the development of AR specific antibodies that could be used in immunohistochemical analysis (Chang *et al.* 1988, Lubahn *et al.* 1988, Trapman *et al.* 1988). Once these tools became available, several laboratories reported that the AR is expressed not only in tissues that were known to be sensitive to androgens but in multiple tissues that were not thought to be androgen regulated (Ruizeveld de Winter *et al.* 1991, Kimura *et al.* 1993). These studies demonstrated that AR was expressed widely throughout the body including in the liver, gastrointestinal tract, and the bladder. Interestingly, AR was expressed exclusively in the nucleus, suggesting that it could be functioning as a transcription factor thus regulating cellular processes (Kimura *et al.* 1993).

Studies in animal models have been instrumental in defining the role of AR in bladder physiology. In the lower urinary tract of male rats, the AR is present in the

urothelium, bladder smooth muscle cells, proximal urethra striated muscle cells, and neurons in the autonomic ganglia of the prostatic plexus suggesting that androgens could have a direct effect in the lower urinary tract (Salmi *et al.* 2001). In a study of juvenile male rats subjected to bilateral orchiectomy or bilateral orchiectomy followed by supplementation with testosterone, the presence of testosterone was associated with increased bladder mass and increased bladder smooth muscle mass, supporting the notion that hormones have a role in the development of these tissues (Shortliffe *et al.* 2014). The rat model was also used to study the effect of androgens on the autonomic neurons that supply pelvic visceral organs. In the castrated animals, noradrenergic pelvic neurons that supply the vas deferens, prostate gland, urinary bladder, and colon were only ~60% the size of those in intact control animals (Keast & Saunders 1998). From the previously mentioned studies, we can conclude that the AR and androgens have a role in the development and physiology of bladder tissue in males. These studies make it plausible that altered AR expression and function may be involved in the etiology and progression of bladder cancer.

Role of AR in bladder carcinogenesis

Radical cystoprostatectomy is one surgical approach for male patients with muscle invasive bladder cancer and includes removal of the prostate. Several studies have evaluated the prostatic tissue from these surgeries and found prostate cancer or high grade prostatic interstitial hyperplasia was detectable in ~30–50% of the tissues (Alsinnawi *et al.* 2012, Fritsche *et al.* 2012, Nevoux *et al.* 2012, Bruins *et al.* 2013, Ahmadi *et al.* 2014). While most of the prostate cancers were low grade, one large study reported that 22% were clinically significant (Bruins *et al.* 2013). Most bladder cancer patients are of advanced age, hence it is not altogether surprising that there was a high incidence of low grade prostate cancer. However, it is notable that one study reported the presence of prostatic carcinomas, particularly carcinoma with stromal involvement, was associated with a worse prognosis (Bruins *et al.* 2013). This observation suggested that a common factor or factors contribute to both malignancies. It is arguable that the AR, a pivotal molecule in the development and progression of prostate cancer, may be this factor.

In an effort to determine the role of the AR in bladder cancer, several labs have demonstrated, using animal models, that alteration of AR expression and signaling affect tumor formation. Early studies utilized

N-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) to induce tumors in rats in the presence or absence of testosterone (Okajima *et al.* 1975). This study determined that testosterone promoted bladder carcinogenesis in female rats (Okajima *et al.* 1975). Interestingly, this same group found that the anti-estrogen diethylstilbestrol inhibited carcinogenesis and progression of bladder tumors in male rats (Okajima *et al.* 1975). Studies which utilized *N*-nitrosobis(2-oxopropyl)amine (BOP) to induce prostate tumors in rats revealed that testosterone promoted urothelial carcinoma formation, and conversely, orchiectomy reduced the incidence of bladder tumor formation (Pour & Stepan 1987). Further analysis demonstrated that BBN-induced bladder carcinogenesis could be altered by modifying testosterone signaling using luteinizing hormone-releasing hormone (LH-RH) analogues (Matsuki *et al.* 1989, 1992). Since BBN was shown not to effect testosterone levels, the LH-RH analogues are responsible for observed changes in hormone levels (Matsuki *et al.* 1989). In female rats, testosterone administration promoted bladder tumor formation in intact rats but not in oophorectomized rats, suggesting that testosterone signaling in conjunction with estrogen signaling play a role in bladder cancer formation (Terada *et al.* 1992).

A robust study tested not only castration and LH-RH agonists but also the AR antagonist flutamide and the 5 α -reductase (an enzyme that converts testosterone to 5 α -dihydrotestosterone) inhibitor Finasteride (Imada *et al.* 1997). Flutamide was shown to inhibit bladder tumor incidence in a dose dependent manner while Finasteride showed no difference. This result argues that testosterone might have a potent effect on bladder carcinogenesis but that conversion of testosterone to the more active form, 5 α -dihydrotestosterone, does not occur in this model.

Bypassing the need for chemical induction of tumors, Johnson *et al.* (2008) demonstrate similar findings to the preceding using a transgenic mouse model of bladder tumorigenesis. UPII-SV40T mice express the SV-40 T antigen (tumor virus protein that inhibits functioning of p53 and pocket proteins) exclusively in the urothelium and reliably develop bladder tumors (Johnson *et al.* 2008). Using this model, the authors show that castration inhibits tumor growth (Johnson *et al.* 2008).

The previous lines of evidence support the role of testosterone in bladder carcinogenesis, but fail to demonstrate that it is AR mediated. One study found that unilateral orchiectomy actually promoted BBN induced bladder carcinogenesis in male rats (Saikawa *et al.* 2001). Although these data seem contradictory to previous reports, the study noted that AR protein expression was

higher following unilateral orchiectomy (Saikawa *et al.* 2001). Thus, it is possible that despite orchiectomy, increased AR levels could compensate for decreased testosterone levels. Increased AR expression is one mechanism that results in continued proliferation of prostate cancer cells in castrate levels of androgen (Devlin & Mudryj 2009). Thus, the increased AR expression in bladder cells could be directly involved in bladder carcinogenesis.

Studies have also directly addressed the importance of AR in bladder cancer development. Miyamoto *et al.* (2007) demonstrated that AR knockout (ARKO) mice do not get BBN induced tumors. Interestingly, they also found that BBN did induce tumors in 25% of ARKO mice supplemented with dihydrotestosterone (DHT) (Miyamoto *et al.* 2007). BBN induced tumors in 50% of castrated WT mice (Miyamoto *et al.* 2007). These results strongly suggest that AR is key in bladder carcinogenesis. The ARKO mice were generated by a disruption of exon 2 of the AR gene, which encodes the second zinc finger of the AR DNA-binding domain. The result that DHT supplementation in the ARKO mice induces tumor formation argue that, i) while an AR molecule that is missing one zinc finger has greatly diminished AR activity, enough residual activity remains, so that supplementation with DHT can partly restore AR signaling or ii) that androgens exert an effect on cells by a mechanism that is independent of AR. In a study where the AR was ablated only in urothelial tissue, bladder tumor development decreased and there was a prolonged survival in animals that developed tumors (Hsu *et al.* 2013). These data differ from those generated using a total body AR KD detailed previously, suggesting that the activity of the AR in other tissues also regulates tumor formation. Furthermore, this study showed that over-expression of the AR in the immortalized but non-transformed urothelial cell line SV-HUC-1 promoted chemically-induced tumorigenesis (Hsu *et al.* 2013). In a β -catenin driven mouse model of bladder tumorigenesis, males developed tumors at a rate of 45% compared to 3% of females (Lin *et al.* 2013). Males showed AR nuclear translocation while females in general showed much weaker AR staining (Lin *et al.* 2013). Further analysis revealed that nuclear translocation of the AR is critical for tumor development in these mice, arguing that an interaction between β -catenin and AR is instrumental in the development of this neoplasia (Lin *et al.* 2013).

Exposure to carcinogens, such as cigarette smoke and industrial chemicals, had been suggested to be a cause for high incidence of bladder cancer in men. UDP-glucuronosyltransferases (UGTs) play a major role in the

elimination of numerous carcinogens by transporting and sequestering these substances (Bock 2010). The urinary bladder is the main pathway for excretion of glucuronides and bladder cells express all the subtypes of UGTs except UGT2B17 (Nakamura *et al.* 2008). In an immortalized urothelial cell line transfected with an AR expression plasmid, treatment with androgens reduced the expression of all UGT1A subtypes and treatment with hydroxyflutamide, an AR antagonist, partly reversed this effect. An analysis of bladder tissues from ARKO mice showed that the expression of all the UGT1A genes were upregulated (Izumi *et al.* 2013). Likewise, castration of male mice resulted in increased UGT1A expression, confirming AR-dependent repression of these detoxification genes (Izumi *et al.* 2013). Expression of UGT1A was lower in human tumor tissue, than in normal urothelial cells, and although not statistically significant, there was an inverse correlation between UGT1A and AR expression (Izumi *et al.* 2013). These data suggest that AR-mediated repression of UGTs may decrease the inactivation of carcinogens and thus could play a role in promoting bladder tumorigenesis.

Together, the previously reviewed studies strongly indicate that AR is a participant in bladder tumorigenesis. Much of the presented data suggests that androgen signaling promotes the formation of both male and female bladder tumors. These data are consistent with two possible hypotheses regarding male vs female bladder cancer incidence; i) Males have higher AR expression or ii) higher levels of circulating androgens predispose to bladder cancer (Izumi *et al.* 2014).

Expression of the AR in human bladder cancer

In recent years, a number of studies sought to define the expression of AR in human bladder cancers and to determine if differences in expression correlate with various aspects of the disease. Early studies demonstrated that AR was present in bladder cancer tissue and that AR was present at higher levels in tumor tissue than in control tissue suggesting upregulation of AR in malignancy (Laor *et al.* 1985). Subsequent studies supported these results demonstrating that the AR was expressed in bladder tumor tissue and that AR expression is notably higher in tumor tissue than in non-tumor tissue (Noronha & Rao 1986, Zhuang *et al.* 1997, Mir *et al.* 2011, Mashhadi *et al.* 2014, Mohanty *et al.* 2014, Williams *et al.* 2015). An analysis using western blots showed that in addition to the full length AR, other AR isoforms (90 and 60 kD) could be detected in tumors (Zhuang *et al.* 1997). Thus, AR has been

detected in bladder cancer tumors in a number of studies but the contribution of AR expression to the progression of this disease is not fully understood.

A study by Mashhadi *et al.* (2014) demonstrated that AR expression positively correlated with tumor stage and grade and that patients with AR-positive tumors had a poorer prognosis than those with AR-negative tumors. This study also reported that AR-positivity is associated with metastasis. An analysis of non-metastatic and metastatic tumors found that a higher percentage of metastatic lesions expressed AR than primary tumors (Miyamoto *et al.* 2012). Additional studies reported that AR expression is detected in a higher percent of invasive tumors (15.1%) vs non-invasive tumors (9%) and that AR expression correlated with tumor progression (Zheng *et al.* 2011). These data suggest that AR expression is positively correlated with disease progression.

In contrast, conflicting studies argue that AR expression actually decreases with increasing stage and grade of disease indicating low AR expression is associated with progression and invasion (Laor *et al.* 1985, Boorjian *et al.* 2004, Tuygun *et al.* 2011, Miyamoto *et al.* 2012, Gakis & Stenzl 2013). One study reported that while AR levels were more often detected in invasive tumors, this did not affect tumor recurrence and did not correlate with time to death or death from disease (Mir *et al.* 2011). Of note, it was shown that carcinoma *in situ* (CIS) tumors were an outlier possessing lower AR expression than pT1 tumors (Boorjian *et al.* 2004). Interestingly, one study also showed that none of the control tissue samples expressed AR while ~51% of tumors did show expression (Tuygun *et al.* 2011). This suggests that although AR may decrease with disease progression, AR upregulation may occur in certain stages of tumorigenesis. However, the overall contribution of AR expression to bladder cancer remains controversial.

Some investigations sought to determine if AR expression is significantly different in male tumors vs female tumors. One study found that AR levels in male tumor tissue were higher than that found in female tumor tissue suggesting a mechanism that explains the discrepancy in the incidence of bladder cancer in males and females (Laor *et al.* 1985). This data is supported by the study of β -catenin induced bladder tumorigenesis where AR was more highly expressed in male mouse tumors and shown to factor into tumor progression (Lin *et al.* 2013). Additionally, studies in some female patients revealed that the Xq11-12 locus containing the active AR gene is deleted in some tumors and X chromosome inactivation occurs in others (Cheng *et al.* 2004). Thus, although AR expression and signaling may predispose men to bladder cancer over

females, AR expression may be less involved in bladder tumorigenesis in females than in males (Cheng *et al.* 2004). The previous studies are challenged by more recent studies which argue that the AR was expressed at similar levels in both male and female patients, and that there was no statistically significant difference in AR expression between male and female tumors (Laor *et al.* 1985, Mir *et al.* 2011, Tuygun *et al.* 2011, Mashhadi *et al.* 2014). Therefore, the role of the AR in male vs female bladder tumors is unresolved.

In summary, the AR has been detected in human bladder cancer in a number of studies but there is no clear consensus on whether its expression level is important in bladder cancer progression. The data overall suggest that AR expression is frequently upregulated in early stages of bladder cancer but sometimes decreases as the disease progresses. Bladder cancer, like other cancers, is a heterogeneous group of diseases and dependence on AR for later stages of disease may vary from patient to patient. The AR does not appear to be a good bladder cancer prognosticator at this time. Whether the AR makes a viable therapeutic target may depend on stage and the particular patient's unique tumor.

The data suggests that AR does not differentiate between male and female tumors. One hypothesis that resolves these discrepancies is that androgen signaling, rather than AR levels, is primarily responsible for the discrepancy between male and female incidence of bladder cancer (Izumi *et al.* 2014). Although the AR can be variably expressed and seems to play a role in the disease, males have higher circulating androgen than do females. Therefore, the high level of circulating androgen, and thus AR activity, may be central to predisposing males to bladder cancer. However, it is most likely that some combination of elevated androgen signaling and AR levels play a role in this disease. In the following sections, we will delve into the literature pertaining to the mechanistic functions of the AR in bladder cancer and examine the therapeutic implications.

AR, co-regulators, and collaborating signaling pathways in bladder cancer

Several studies investigated the expression of known AR co-regulators and their clinical significance. Previous studies of AR coregulators were conducted in the context of prostate cancer and the results differed in bladder tumor studies (Boorjian *et al.* 2009). AR and its coregulators NCOA1, NCOA2, NCOA3, CREBBP, and EP300 were expressed at varying degrees in both bladder tumor

samples and in the TCC-SUP and UM-UC-3 UCCB cell lines (Boorjian *et al.* 2009). Interestingly, it was determined that siRNA-mediated inhibition of several co-factors investigated decreased viability of bladder cancer cells (Boorjian *et al.* 2009). However, the study noted that only NCOA1 levels were significantly lower in tumor tissue vs control tissue (Boorjian *et al.* 2009). Consistent with previous studies, this analysis also reported that AR trended toward upregulation in tumor tissue (Laor *et al.* 1985, Boorjian *et al.* 2009, Miyamoto *et al.* 2012, Mashhadi *et al.* 2014). Despite this trend, AR was also found to decrease with increasing stage of disease (Boorjian *et al.* 2009).

An investigation of AR expression along with two chromatin modifying coregulators, lysine-specific demethylase 4A (JMJD2A) and lysine-specific histone demethylase 1A (LSD1) showed that both AR and JMJD2A expression decreased in tumor tissue when compared to benign tissue, while LSD1 levels increased (Kauffman *et al.* 2011). However, all three proteins were found to decrease with progression of the disease (Kauffman *et al.* 2011). JMJD2A expression additionally correlated with lymphovascular invasion, concomitant CIS, tobacco usage, and decreased overall survival (Kauffman *et al.* 2011).

Cyclin D1, the regulatory subunit of the cyclin D1/CDK4/6 kinase complex, phosphorylates RB to promote cell cycle progression. However, in the context of prostate cancer, the interaction of cyclin D1 with AR inhibits AR activity (Petre-Draviam *et al.* 2003). This effect of cyclin D1 on AR has also been detected in T24 bladder cells, suggesting that this interaction is not prostate specific (Petre-Draviam *et al.* 2003). A meta-analysis of 15 studies on cyclin D1 expression and bladder tumorigenesis revealed that increased cyclin D1 levels significantly associated with progression-free survival (Ren *et al.* 2014). A cyclin D1 splice variant, cyclin D1b, has been found to be oncogenic in prostate cancer and regulates a transcriptional network that in concert with the AR, enhances proliferation and invasion (Augello *et al.* 2013). At this time, it is unknown if bladder tumors express this cyclin D1b splice variant and if it functions in a similar manner to promote bladder tumorigenesis.

The AR works in concert with other signaling pathways to promote tumorigenesis, often directly interacting with key molecules. β -catenin has been shown to correlate with tumor progression and poor clinical outcomes in patients with bladder cancer, and immunohistochemical studies of bladder tumors identified a strong correlation between nuclear AR expression and β -catenin (Li *et al.* 2013).

Androgen treatment of AR-expressing cells promoted β -catenin translocation into the nucleus and its transcriptional activity. Co-immunoprecipitation studies indicated that in bladder tumor derived cell lines, β -catenin physically interacts with the AR (Li *et al.* 2013).

The AR interacts with the EGFR signaling pathway to exert an effect on bladder tumor cell physiology. An immunohistochemical study of bladder tumors showed a strong association between expression of AR and the EGFR, phospho-EGFR, and ERBB2 (Zheng *et al.* 2011). Further analysis in AR expressing bladder tumor derived cell lines indicated that treatment with androgens resulted in increased expression of EGFR and ERBB2 at the mRNA and protein levels (Zheng *et al.* 2011). Conversely, shRNA mediated AR ablation reduced EGFR and ERBB2 levels (Zheng *et al.* 2011). Additionally, treatment of the cells with androgens increased phosphorylation of EGFR and its downstream targets AKT and ERK1/2, suggesting that AR may have a role in activating the EGFR signaling pathway (Zheng *et al.* 2011). Further studies on the interaction between AR and EGFR found that the EGFR inhibitor PD168393 antagonized the effect of androgen treatment on AR transcriptional activity (Hsieh *et al.* 2013). Treatment of cells with an AR antagonist abolished the effects of both proteins (Hsieh *et al.* 2013). EGF treatment promoted the association of AR with estrogen receptor- β or with SRC, suggesting that EGFR activates AR by promoting complex formation (Izumi *et al.* 2012). These studies argue that there is a complex reciprocal regulation between the EGFR and AR signaling pathways and that targeting both may be efficacious in the treatment of certain malignancies.

Clearly, the AR functions in a number of ways and interacts with many pathways. The data previously presented regarding β -catenin signaling, cyclin-d1 interactions, and growth factor cross-talk with the AR bring up the interesting hypothesis that cell-cell communication in the tumor microenvironment may be instrumental in altering AR signaling in bladder cancer. One study performed by Turkeri *et al.* (1998) demonstrates that EGF and TGF- α were expressed in the stroma and around vessels found within tumor samples. Thus, it is possible that factors derived from other cell types may influence AR signaling to promote tumor progression. However, this notion requires further study.

The AR, genetic variation, and bladder cancer

Genomic instability of the AR gene has been detected in bladder cancer and data suggest that this may be involved

in disease progression. A highly polymorphic trinucleotide (CAG) repeat, or polyglutamine expansion (polyGln), is present in the AR gene (Allen *et al.* 1992). Investigation of the role of the polyGln region has shown that its size inversely correlates with AR transactivation function (Kazemi-Esfarjani *et al.* 1995). Moreover, studies have demonstrated that the polyGln region in the AR gene locus can have variable lengths in different tumors (Gonzalez-Zulueta *et al.* 1993). In one Ta grade II tumor, this locus was expanded while in more advanced tumor tissue, this locus was shortened (Gonzalez-Zulueta *et al.* 1993). These data indicate that changes to this region may impact this disease and that shorter repeats, and thus increased AR transactivity, may be involved in disease progression. Building on this data, Teng *et al.* (2010) examined 95 male patients with urothelial carcinoma and found that their polyGln region was significantly shorter than those found in a reference group. These results further support the notion that a shorter polyGln region, and increased AR activity, is involved with bladder cancer etiology and progression.

Several hypotheses can be generated regarding the AR's contribution to bladder cancer based on genetic variation studies. Genome wide association studies (GWAS) have identified a number of small nucleotide polymorphisms (SNPs) which are associated with increased risk of bladder cancer. A number of these SNPs are next to or within genes that have previously been associated with cancer (Golka *et al.* 2011). Interestingly, several SNPs map to 8q24, a region of the genome that has been described as a gene desert (Huppi *et al.* 2012). Notably the Myc gene lies at the border of this region (Huppi *et al.* 2012). The rs9642880 T allele, which has been shown to be associated with an increased risk of bladder cancer in North European and Chinese populations (with an odds ratio of 1.22 and 1.64, respectively), maps to this region (Kiemeny *et al.* 2008, Wang *et al.* 2009). The SNP lies within the non-protein coding BC042052 gene (aka CASC11, CARLo-5) that has been associated with certain cancers, hence this RNA transcript may contribute to bladder tumorigenesis (Luo *et al.* 2014, Zhang *et al.* 2014). The Myc enhancer region physically interacts with the active regulatory region of the CARLo-5 promoter, where long-range interaction of Myc enhancer with the CARLo-5 promoter regulates CARLo-5 expression (Kim *et al.* 2014). Previous studies have shown that the AR directly controls Myc transcription (Gao *et al.* 2013). Additional studies found that Myc expression is elevated in bladder cancers (Schmitz-Drager *et al.* 1997). Therefore, the AR may be

directly or indirectly affecting the transcription of this non-coding gene by controlling Myc transcription.

Two independent studies have linked a variation within the prostate stem cell antigen (PSCA) gene with increased susceptibility to bladder cancer (Wu *et al.* 2009, Fu *et al.* 2012). The rs2294008 SNP cancer predisposing variant alters the start codon which results in a nine amino acid N-terminal truncation (Wu *et al.* 2009). The risk allele was associated with increased PSCA mRNA expression in two sets of bladder tumor samples arguing that enhanced PSCA expression contributes to bladder tumorigenesis (Fu *et al.* 2012). Previous studies have shown that PSCA is a direct target of AR therefore AR-dependent increased PSCA could contribute to tumorigenesis (Jain *et al.* 2002). Further studies are needed to explore this possibility.

Multiple studies have identified bladder cancer susceptibility SNPs in the urea transporter gene, SLC14A1 (Garcia-Closas *et al.* 2011, Rafnar *et al.* 2011, Singh *et al.* 2014, Matsuda *et al.* 2015). Additional studies found that the rs10775480 risk allele is associated with a significant decrease in urine concentration (Koutros *et al.* 2013). The data suggest that this urea transporter has the ability to influence urine concentration and ultimately bladder cancer susceptibility. The authors hypothesized that bladder cancer risk might be related to this impaired urine concentrating ability. The SLC14A1 gene is an AR regulated gene that is decreased in prostate cancer tissue hence AR-dependent decrease of SLC14A1 could contribute to bladder carcinogenesis (Vaarala *et al.* 2012).

The role of the AR in bladder cancer invasion and angiogenesis

The AR may also contribute to invasive and metastatic disease. CD24, a glycosyl phosphatidylinositol-linked sialoglycoprotein, contributes to tumor growth, metastasis, and is associated with poor outcome in bladder tumorigenesis. The BBN animal model of bladder tumor induction was used to define the importance of CD24 in tumor formation and metastasis. Ablation of CD24 resulted in fewer metastases than in control animals. Interestingly, this result was restricted to male mice, suggesting a role for androgens (Overdevest *et al.* 2012). In cell culture studies, an AR knockdown resulted in decreased CD24 expression and cell proliferation, while treatment with androgens increased CD24 levels, arguing that CD24 is androgen regulated in urothelial cells (Overdevest *et al.* 2012). Others have found that androgen stimulation of AR positive bladder cancer cells promoted a mesenchymal phenotype, migration and invasion *in vitro*,

and metastasis *in vivo* (Jing *et al.* 2014). Furthermore, it was determined that this effect was mediated via canonical Wnt signaling which activates Slug (Jing *et al.* 2014). These data were supported in a subsequent study that demonstrated that AR mediated promotion of migration (Jitao *et al.* 2014).

Angiogenesis is an important feature of tumor development. Thrombospondin-1 (TSP1) is a secreted anti-angiogenic protein that forms a 450-kDa homotrimer (Armstrong & Bornstein 2003). Studies in the UPII-SV40T mouse model of bladder tumorigenesis showed that castration increased TSP1 in bladder tissue (Johnson *et al.* 2008). Additionally, studies of human bladder tissue indicated that TSP1 expression is lower in malignant tissue than in non-malignant tissue in the same individual (Johnson *et al.* 2008). These data argue that one role of the AR in bladder tumorigenesis may be repression of TSP1.

Therapeutic potential of targeting the AR in bladder cancer

One currently effective treatment for NMIBC is intravesical administration of attenuated mycobacterium bacillus Calmette-Guerin (BCG) which elicits an anti-tumor immune response (Malkowicz 2000). Many patients respond favorably to this treatment (Chen *et al.* 2003). However, some patients prove to be non-responders and many others relapse following treatment (Chen *et al.* 2003). Understanding why patients fail to respond and why others relapse could provide opportunities for novel interventions. It is thought that the effectiveness of BCG treatment is in part dependent on its adherence to the urothelial wall via an interaction with $\alpha 5 \beta 1$ integrin (Zhang *et al.* 2000). Furthermore, it has been shown that BCG treatment results in the upregulation of IL6 which promotes expression of $\alpha 5 \beta 1$ integrin thus allowing BCG to promote its own efficacy (Bevers *et al.* 1998, Zhang *et al.* 2000). Chen *et al.* (2003) demonstrate that androgens inhibit BCG-induced expression of IL6 in the UCCB cell lines 253J and T24. Furthermore, these authors demonstrate that the use of the anti-androgens bicalutamide and hydroxyflutamide can reverse the effects of androgen on inhibition of IL6 expression (Chen *et al.* 2003). Thus, targeting AR signaling could improve patient responses to BCG treatment.

Efficacy of androgen deprivation therapy (ADT) in attenuating bladder cancer recurrence comes from a study of prostate cancer patients who also had primary bladder cancer of varying grades and stages ranging from Ta to beyond T1 (Izumi *et al.* 2014). Retrospective analysis of

patients who did and did not receive ADT for prostate cancer revealed that men given ADT had longer 5 year recurrence free survival rates and had fewer subsequent recurrences (Izumi *et al.* 2014). This analysis also found ADT was an independent prognosticator of bladder cancer recurrence and that the effect of ADT on recurrence is time dependent indicating longer treatment periods provide more patient benefit (Izumi *et al.* 2014). Based on the work presented here, ADT could become a novel way to treat and better prevent recurrence of bladder tumors. Clinical trials are being planned to assess these possibilities (Izumi *et al.* 2014). This work also suggests that ADT could be effective in treating a range of tumor grades and stages. Whether ADT leads to a decrease in progression of the disease remains to be tested. The BCG data presented earlier leads to the speculation that targeting the AR in early stage bladder cancer along with BCG therapy could lead to even further reduced rates of recurrence. However, this potential treatment strategy needs to be investigated.

Other investigators have also demonstrated the utility in targeting the AR for bladder cancer therapy. Inhibition of AR signaling via castration, small molecule drugs, or combinations of these resulted in decreased proliferation, tumor volume, and tumor weight while also increasing cell death *in vivo* (Miyamoto *et al.* 2007). Moreover, there was decreased expression of bFGF, VEGF, and MMP-9, all documented oncogenes in the context of bladder cancer (Miyamoto *et al.* 2007). *In vitro* models utilizing the AR positive cell lines TCCSUP and UM-UC-3 and the AR-negative cell line 5637 demonstrated that AR targeting siRNA can also attenuate proliferation of bladder cancer cells but only in AR positive lines (Miyamoto *et al.* 2007). Interestingly, they found that AR siRNA was more effective than hydroxyflutamide (Miyamoto *et al.* 2007). Additional studies showed that siRNA-mediated AR inhibition increased apoptosis and decreased proliferation and migration in UCCB cell lines (Wu *et al.* 2010). This was coincident with a decrease of cyclin D1, BclXL, and MMP9 (Wu *et al.* 2010). *In vivo* AR inhibition attenuates tumor growth of T24 xenografts (Wu *et al.* 2010). It was also determined that AR inhibition sensitized UM-UC-3 cells to doxorubicin treatment (Shiota *et al.* 2012). Drawing from these studies, it is reasonable to hypothesize that targeting the AR could prove to be an efficacious treatment not only for early stage disease but also for advanced tumors expressing the AR. Furthermore, one study demonstrated that AR targeting could sensitize bladder tumors to chemotherapy suggesting a rationale for the use of AR antagonists to augment treatment of advanced stage disease.

In addition to more traditionally considered means of targeting the AR for therapy, alternative approaches could prove useful. Given the large number of signaling pathways that intersect with the AR reviewed in a previous section, targeting the AR-interacting components and signaling pathways may indirectly affect AR activity. Targeting of several AR coregulators was shown to inhibit UCCB cell viability suggesting this could be an effective strategy (Boorjian *et al.* 2009). It was determined that pharmacological inhibition of LSD1, an AR co-regulator mentioned previously which is upregulated in bladder cancer, attenuated viability of bladder cancer cells and decreased androgen-mediated transcription (Kauffman *et al.* 2011). Other approaches for reducing AR signaling include the utilization of natural compounds found in certain foods that may possess anti-cancer properties. One such compound is silymarin, a flavonoid found in the milk thistle plant (Zhu *et al.* 2001). It has been demonstrated that silymarin and its primary constituent silibinin can inhibit the activity of androgen signaling and prevents AR nuclear localization in prostate cancer cells (Zhu *et al.* 2001). It is worth investigating if these findings can translate to bladder cancer for similar therapeutic purposes.

Although there is much evidence that targeting the AR for bladder cancer therapy could prove beneficial, it is important to keep in mind side effects that could ensue from such treatments. Common side effects of ADT include loss of libido, erectile dysfunction, and hot flashes (Higano 2003). Although manageable, these effects can prove rather distressing for suffering patients. More serious side effects include physical symptoms such as gynecomastia as well as the exacerbation of other conditions such as hypertension and diabetes (Higano 2003).

Long term outcomes of a new therapy must also be carefully considered. In the context of prostate cancer, ADT has been shown to promote EMT (Sun *et al.* 2012). Since studies suggest that the AR and androgen signaling promote EMT, migration, and invasion in bladder tumors and flutamide or an siRNA targeting the AR inhibits these responses, it is reasonable to hypothesize that ADT could be beneficial in the long term for certain patients (Jing *et al.* 2014, Jitao *et al.* 2014). However, identifying the patient cohort that would benefit from this intervention should be addressed prior to initiating such therapies.

Final discussion and conclusions

The work reviewed supports the hypothesis that bladder cancer is in fact an endocrine-related cancer in which AR

and androgen signaling play a role in the etiology and progression of the disease. This is not all that surprising given the work highlighting the role of androgen signaling in normal bladder function. This hypothesis provides, at least in part, a reasonable explanation for the glaring disparity of bladder cancer diagnoses between males and females. Scientists and clinicians could begin to view bladder cancer in a new light and traditional therapies used to treat prostate cancer could be used to treat bladder tumors as well. However, questions abound in this burgeoning field requiring additional studies.

A number of studies have demonstrated that the AR is expressed in bladder tumors and is upregulated in comparison to normal tissue. However, significant evidence suggests an inverse relationship between AR expression and advanced disease. AR expression could be biphasic during the course of this disease. This raises the interesting question of when to target the AR for effective bladder cancer treatment and in which patients. Future work must address this question to determine the best time to intervene in this manner. Whether targeting the AR could potentially lead to eventual tumor progression in those patients exhibiting the inverse relationship must be addressed.

A number of studies describe the ways of which the AR promotes bladder cancer progression. AR signaling in bladder cancer affects a number of key facets of the disease including proliferation and migration. Furthermore, the AR and AR signaling can interact with a number of key pathways described in these diseases. This raises the possibility of interesting combination therapies that could be tried. Bladder cancer is a disease with few treatment options and no approved targeted therapies (Cancer Genome Atlas Research Network 2014). Targeting the AR and possibly combining it with other drugs could prove to be a more effective treatment strategy.

Other questions arise when considering whether AR targeting should be adopted as a method to treat bladder malignancies. Would tumors eventually become resistant to such therapies giving rise to castration-resistant bladder cancer (CRBC) similar to castration-resistant prostate cancer (CRPC)? What would such a disease state look like? Would it look like CRPC or be a completely different entity? What mechanisms might drive CRBC and how would this state be overcome?

In conclusion, we have explored the body of evidence that suggests the AR is a participant in bladder tumorigenesis and thus represents a potential target for bladder cancer treatment. However, more work is needed to understand the unique features of AR signaling in bladder

tumor cells to determine the efficacy in targeting the AR for treatment of this malignancy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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