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The Role of the Androgen Receptor in the Development and Progression of Bladder Cancer

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Men are at a higher risk of developing bladder cancer than women. Since bladder cancer cell lines and tissues were found to express the androgen receptor, efforts have been made to inspect whether androgen-mediated androgen receptor signals are implicated in bladder carcinogenesis as well as cancer progression. Mounting evidence supports the view that bladder cancer is a member of the endocrine-related tumors and may clearly explain the gender-specific difference in the incidence. However, the underlying mechanisms of how androgen receptor signals regulate bladder cancer growth are still far from fully characterized. Moreover, it remains controversial whether the androgen receptor pathway always plays a dominant role in bladder cancer progression. In this review, we summarize the available data on the involvement of androgen receptor signaling in bladder cancer. In particular, current evidence demonstrating the stimulatory effects of androgens on tumor progression or, more convincingly, tumorigenesis via the androgen receptor pathway may offer great potential for androgen deprivation as a therapeutic or chemopreventive option in patients with bladder cancer.

Key words: androgens – androgen receptor – bladder cancer – carcinogenesis – progression

INTRODUCTION

Bladder cancer, mostly urothelial carcinoma, is the second most common genitourinary malignancy, leading to significant morbidity and mortality (1–4). Unlike most epithelial tumors, divergent pathways of tumorigenesis are involved in urothelial carcinoma (2–5). These separate mechanisms result in different biological behaviors and phenotypic variants, which gives rise to at least two distinct clinicopathologic types of neoplasms: non-invasive low-grade tumor and high-grade, often invasive, carcinoma. Patients with low-grade tumor generally have a favorable prognosis after transurethral tumor resection with or without intravesical pharmacotherapy, but they carry a lifelong risk of frequent recurrence (50–70%) with occasional progression to invasion. Those with the other, high-grade muscle-invasive tumor, even when given radical cystectomy with or without systemic chemotherapy, are at a high risk for metastasis. Interestingly, owing to the lifelong need for monitoring for recurrence, the typical cost incurred by a bladder tumor patient from diagnosis to death has been reported to be the highest among all cancers (6). In addition, from 1990 to 2006, despite improvements in surgical technique and perioperative care, compared with prostate cancer (39% decrease), bladder cancer mortality was decreased only by 5% (3,7). Thus, identification of the molecules playing a key role in bladder cancer development and progression is urgently needed to improve the diagnosis, treatment and monitoring of the patients.

Comparative studies have demonstrated that men are three to four times more likely to develop bladder cancer than women, while female patients present with more aggressive tumors than male patients (3,4,8). In the USA, there will be

projected deaths of 10 510 men and 4370 women from bladder cancer in 2012, accounting for 18.9 and 24.4% of newly diagnosed cases, respectively (4). Environmental or lifestyle factors, such as industrial chemicals and cigarette smoke, have been blamed for the gender-specific differences in bladder cancer incidence and aggressiveness. However, even after controlling these carcinogenic factors, bladder cancer remains a predominant disease among men (4,9,10). Thus, the exact mechanisms responsible for the gender differences remain unclear. Recent studies have provided data supporting the hypothesis that androgen receptor (AR) signaling plays an essential role in the development and progression of bladder cancer, which may explain some of the differences between male and female tumors.

In this article, we review molecular evidence suggesting the modulation of bladder tumorigenesis and tumor progression through the AR pathway. We also highlight alterations of genetic pathways regulated by androgens in bladder cancer cells, which might contribute to development of prognostic biomarkers and/or novel targeted therapies.

ANDROGEN RECEPTOR

The AR, located on the X chromosome (q11-12), is well-known as a ligand-inducible transcription factor that regulates target gene expression (11–13). As a member of the nuclear receptor superfamily, the *AR* gene consists of eight exons that encode four structurally and functionally distinct domains: the NH_2-terminal transactivation domain, the DNA-binding domain, a hinge region and the COOH-terminal ligand-binding domain (11). The AR mediates its physiological activities by binding to androgens. Testosterone, upon entry into the target cell, binds to the AR directly or after conversion to 5α-dihydrotestosterone (DHT) by 5α-reductases. Non-ligand-bound AR is usually located in the cytoplasm where it associates with heat shock proteins (HSPs). Alternatively, this transcriptionally quiescent AR can be degraded via E3 ubiquitin ligase. The ligand-AR complex induces a conformational change in the AR, resulting in release of the HSPs and translocation of the complex to the nucleus. Sequentially, in a homodimeric fashion, the activated AR binds to the tissue-specific androgen-response element and recruits further proteins, such as general transcription factors and RNA polymerase II, leading to specific transcriptional activation or repression of target genes. In addition, primarily in prostate cancer cells, ligand-independent activation of the AR pathway by, for instance, peptide growth factors such as epidermal growth factor (EGF), has been demonstrated, presumably through signal transduction pathways (12).

Ligand-mediated receptor transactivation can be further modulated by a number of co-regulatory proteins, termed coactivators and corepressors (13). It has been well acknowledged, especially in prostate cancer cells, that the transcriptional activity of AR is dependent on AR-coregulator complex composition. Increased affinity between AR and coregulator is generally associated with ligand binding, which subsequently enhances AR transactivation by facilitating DNA occupancy, chromatin remodeling, ensuring AR protein stability and proper AR subcellular distribution (11–13).

DISTRIBUTION OF THE AR AND THE PHYSIOLOGICAL FUNCTION OF ANDROGENS IN THE BLADDER

The AR is ubiquitously distributed throughout the human body despite gender difference and even in mouse, rat and monkey tissues (14,15). Indeed, a wide range of biological actions of androgens, such as maintaining libido, spermatogenesis, muscle mass and strength, bone mineral density and stimulating erythropoiesis, are known (16). AR expression has also been detected widely in the bladder, including urothelium, muscularis propria (detrusor muscle) and neurons (14,15,17–19).

Increasing evidence from animal and human studies has shown that androgens contribute to urinary tract functions. In studies in male animals, androgens inhibited bladder detrusor muscle contraction via neuronal regulation (20–22). In castrated rat bladder, androgen replacement augmented urothelial thickness, muscle fiber quantity and vessel number (23). Androgen deprivation dramatically down-regulated the activity and expression of tissue enzymes involving cholinergic and non-cholinergic nerve functions (24,25). In a recent study, castration in male rats, via transforming growth factor-β, led to decreases in maximal volume and compliance of the bladder, and androgen supplementation restored bladder dysfunction (26). These findings in animal models suggested that androgens might directly regulate voiding function. In humans, it was suggested that there is a correlation between androgen deficiency and bladder dysfunction (27). A few other studies have shown improvement of lower urinary tract symptoms in men treated with testosterone (28,29).

AR ALTERATIONS IN BLADDER CANCER

Intense efforts have been made to examine the expression of the AR in bladder cell lines and tissue specimens. Table 1 summarizes the results from such studies in human tissue samples and the correlation of AR expression with the clinicopathologic profile of the patients.

Several human bladder cancer cell lines have been found to express the AR at messenger RNA (mRNA) and protein levels (30–34). Using androgen-binding assays in relatively small numbers of tissue specimens, the presence of the AR in bladder cancer was suggested (35,36), although another study failed to show positivity in all the tissues examined (13 bladder and 3 ureter tumors) (37). Subsequently, immunohistochemical analyses have demonstrated that 44–78% of

Table 1. Androgen receptor (AR) expression in bladder cancer and its correlation with clinicopathologic features

Years (reference)	Method	*n*	AR expression												
			Non-tumor	Tumor	*P* value	Gender			Tumor grade			Tumor stage			Prognostic significance
						M	F	*P* value	L	H	*P* value	NMI	MI	*P* value	
1985 (35)	Binding assay	13	17.2 (Fm/mg)	49.5 (Fm/mg)	NA	68 (Fm/mg)	27.7 (Fm/mg)	NA	M: 43.8, F: 27.7 (Fm/mg)	32.4 (Fm/mg)	NA	NA	NA	NA	NA
1986 (36)	Binding assay	6	NA	83%	NA	100%	75%	1.000*	G2: 67%	G3–4: 100%	NA	Ta: 67%	Met: 100%	NA	NA
1990 (37)	Binding assay	13	NA	0%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1997 (38)	IHC	9	NA	78%	NA	100%	33%	0.083*	NA	G2: 50%, G3: 100%	NA	T1: 80%	T3: 75%	1.000*	NA
2004 (39)	IHC	17	0%	52%	NA	NA	NA	NA	NA	G3: 52%	NA	NA	NA	NA	NA
2004 (40)	IHC	49	86%	53%	0.001*	61%	30%	0.104*	89%	49%	0.055*	75%	21%	0.002	NA
			M: 82%	M: 61%	0.101*										
			F: 100%	F: 30%	0.002*										
2007 (31)	RT–PCR	33	NA	100%	NA	NA	NA	NA	NA	NA	NS	100%	NA	NS (Ta *vs.* T1)	Rec: high(+) = higher risk ($P < 0.1$)
2009 (33)	IHC	55	NA	44%	0.06	NA	NA	NA	NA	NA	NA	59%	33%	0.095*	NA
2011 (41)	IHC	139	0%	M: 53%	<0.001*	53%	43%	0.481*	64%	37%	0.002*	60%	21%	<0.001*	Rec: (+) = lower risk ($P = 0.095$); Prog: (+) = lower risk ($P = 0.110$)
2011 (42)	IHC	472	NA	13%	NA	14%	8%	0.159*	12%	13%	0.864*	9%	15%	0.063*	NS
2011 (45)	IHC	93 (UUT)	NA	12%**	NA	NA	NA	NA	NA	NA	0.074*** (L>H)	NA	NA	0.001 (Stage II > I)	NS ($P = 0.568$***)
2011 (34)	IHC	59	84%	Roughly half	<0.001	NA	NA	0.961	NA	NA	NA	NA	NA	0.028 (NMI > MI)	NS
2012 (43)	IHC	188	U: 80%, S: 50%	42%	<0.001 (U), 0.181 (S)	42%	43%	1.000	55%	36%	0.023	51%	33%	0.018	NS (Prog in MI: (+) = higher risk, $P = 0.071$)

M, male; F, female; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; NA, not assessed or not available; NS, not significant; L, low-grade; H, high-grade; NMI, non-muscle-invasive; MI, muscle-invasive; Met, metastasis; Rec, recurrence; Prog, progression; UUT, upper urinary tract tumor; U, benign urothelium; S, benign stroma.
*We calculated the two-tailed *P* value using Fisher's exact test.
**High (>10% of cells stained) nuclear expression.
***High (>10% cells stained) *vs.* low cytoplasmic expression.

bladder tumors express the AR (33,34,38–41). In contrast, in a recent and the largest study involving 492 patients, only 13% of bladder tumors were found to show AR expression (42). Our most recent study (43) showed that AR stained positively in 79 (42%) of 188 bladder tumors. Of note, there was thus a discrepancy in AR positivity between the two studies [i.e. 13% (42) *vs.* 42% (43)] where we stained the bladder tissue microarrays (TMAs) constructed at different institutions, using the same antibody and protocol. As shown in the estrogen receptor expression in breast cancer (44), the type and the duration of tissue fixation may have dramatically altered the levels of immunoreactivity to AR. Indeed, in our previous pilot study, using a quantitative reverse transcription–polymerase chain reaction method, AR signals were detected in all the mRNAs isolated from 33 fresh bladder tumors (31).

In some of the previous studies where control tissues were also assessed (34,40,43), higher rates of AR positivity were observed in benign bladders than in tumors. Conversely, few other studies failed to show higher expression of AR in benign bladder specimens, compared with tumors (33,35,39,41). To date, no studies have shown a statistically significant difference in AR expression between tumors from men and women (34,36,38,40–43). Interestingly, in a study (40), a statistically significant decrease in AR levels was seen in female tumors, but not in male tumors. Most of the studies demonstrated a significant decrease in AR expression in higher grade and/or stage tumors (33,34,40,41,43,45). However, in the largest study (42), there was no significant difference between low-grade (12%) and high-grade (13%) tumors. In addition, in other studies, no significant difference in AR expression between different stages of tumors (38) or even statistically significant higher expression levels in more advanced tumors (42,45) were observed. Moreover, no statistical significance in AR positivity as a prognosticator has been reported. We showed that AR positivity tended to correlate with recurrence in superficial tumors (31) or progression in muscle-invasive tumors (43), while others either failed to show any tendency (34,42,45) or showed an opposite tendency (41).

Thus, the available data obtained by immunohistochemistry regarding the correlation of AR expression in bladder cancer with tumor characteristics remain controversial. This may result from different methods of tissue preparation (e.g. preservation in fixative, embedding in paraffin, TMA construction and sectioning) and staining (e.g. antibody, protocol and criteria for positivity). Nonetheless, predominant results have suggested significant decreases in AR expression in bladder cancer compared with benign urothelium and in high-grade/invasive tumors compared with low-grade/superficial tumors. Further studies are warranted to determine the actual frequency of AR expression and its significance in differences in tumor aggressiveness (e.g. tumor grade, stage, size and multiplicity) and patients' outcome as well as other factors of the patients such as age, gender and history of smoking that is known as a risk factor.

In our recent immunohistochemical study (43) described earlier, benign stromal cells in approximately half of the bladder cancer cases were also found to express the AR. Indeed, stromal AR in the prostate has been shown to play a key role in its carcinogenesis and cancer progression (46). In bladder cancer, several proteins originated from stromal cells as well as tumor–stroma interactions are known to contribute to its growth (47,48). However, to our knowledge, no attempt has been made to elucidate the role of stromal AR in bladder cancer.

In addition to differential AR expression, genetic alterations involving *AR* gene have been described in bladder cancer. Allelic loss of the AR locus was identified in all the three informative cases of female muscle-invasive tumors, but not in corresponding non-neoplastic tissues from the same section of cystectomy (49). A recent study involving 95 male patients with bladder cancer demonstrated a significantly shorter CAG (glutamine) repeat length in exon 1 of the *AR* gene (mean: 20.0), predictive of higher transactivation activity, compared with 95 control males (mean: 21.1) (50). In an earlier study (51), men and women who had 23 and 44 (cumulative) CAG repeats, respectively, were also found to have a significantly elevated risk of urothelial carcinoma, compared with those with longer CAG. The sequencing of mRNAs from two human bladder cancer cell lines revealed a wild-type AR sequence with short CAG repeat lengths (20 in UMUC3 and 22 in TCC-SUP) (33). All these findings suggest that *AR* gene alterations are involved in bladder tumorigenesis.

AR COREGULATORS AND BLADDER CANCER

As described, androgen-mediated AR transcription can be further activated by coactivators. In prostate cancer, up-regulation of various AR coactivators has been observed during tumorigenesis and cancer progression, and deregulated expression of many of these coactivators has been shown to correlate with poor prognosis (12,13,52). AR coregulators have also been investigated in bladder cancer cell lines and tissue samples. First, overexpression of a general steroid hormone receptor coactivator AIB1/SRC-3 was detected in approximately one-third of bladder cancer, which significantly correlated with higher grade/stage and poorer prognosis (53). Second, the expression of AR coactivators, including NCOA1, NCOA2, NCOA3, CREBBP and EP300, was detected in AR-positive bladder cancer cell lines as well as 44–100% of bladder cancer tissue samples even in some of which AR was lacking (33). Among these five coactivators, only NCOA1 showed a significant decrease in its expression levels in tumors, compared with non-neoplastic urothelium. Nonetheless, small interfering RNA (siRNA)-mediated knockdown of any of the coactivators led to marked decreases in androgen-induced proliferation of bladder cancer cells. Remarkably, expression levels of these AR coactivators in bladder cancer cells were not correlated

with AR status nor affected by androgen treatment, suggesting alternative mechanisms of coregulator functions in urothelial carcinoma *vs*. in other AR-positive malignancies such as prostate cancer. Finally, the expression of JMJD2A and LSD1 that mediate AR transcription via histone lysine-demethylation mechanisms was significantly reduced in muscle-invasive bladder cancer, although JMJD2A and LSD1 levels were significantly lower and higher, respectively, in malignant versus benign urothelium (34). Loss of JMJD2A was correlated with worse overall survival ($P = 0.033$), but not with disease-free ($P = 0.409$) or cancer-specific ($P = 0.761$) survival. Furthermore, LSD1 inhibitors suppressed cell proliferation and androgen-induced expression of the AR-regulated neutral endopeptidase (*NEP*) gene in AR-positive bladder cancer lines. Thus, the current data may not only help in identifying AR coregulators as novel prognostic markers but also suggest the involvement of the AR-coregulator complex in bladder carcinogenesis and cancer progression.

AR SIGNALING IN BLADDER TUMORIGENESIS

Because the urothelium is primarily derived from the urogenital sinus during embryogenesis, which in males also gives rise to the prostate, similar mechanisms of AR regulation may exist in the bladder and prostate. The available data on AR alterations between malignant and benign urothelium also support the involvement of AR signals during the development of bladder cancer.

N-Butyl-*N*-(4-Hydroxybutyl) Nitrosamine in Animal Models

Industrial chemicals such as aromatic amines, compounds found in tobacco and tobacco smoke such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and arsenic are well-known bladder carcinogens (54,55). In experimental rodents, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), which can efficiently induce bladder tumors from urothelial dysplasia and carcinoma *in situ* to invasive carcinoma (54), has been commonly used as a suitable model for bladder tumorigenesis.

Using BBN and animal models, others and we have assessed the role of androgens and/or AR signals in bladder carcinogenesis (31,56–59). Consistent with epidemiological findings of male dominance in human bladder cancer (3,4), BBN was shown to more frequently and/or more rapidly induce bladder tumors in male animals than females (31,56,57). In male animals, surgical (bilateral orchiectomy) or medical (diethylstilbestrol or luteinizing hormone-releasing hormone agonist injection) castration, as well as administration of an AR antagonist flutamide, reduces or retards the occurrence of BBN-induced bladder cancer (31,57,58). However, a 5α-reductase inhibitor finasteride showed marginal inhibitory effects on bladder tumor development (58), suggesting that testosterone itself may be a potent promoter of bladder carcinogenesis. Unilateral orchiectomy also led to a significantly higher incidence of bladder cancer, possibly because of a transient increase in the level of testosterone produced from the contralateral testis (59). In female animals, administration of testosterone with or without castration (bilateral ovariectomy) increased the bladder tumor incidence (57). Using an AR-knockout (ARKO) mouse model, we further showed that lack of a functional AR completely prevented bladder cancer development (31), suggesting that AR signals are essential for promoting bladder carcinogenesis. Unexpectedly, a subset of BBN-treated ARKO males supplemented with DHT eventually developed bladder cancer (31). Thus, our results showing the differences in cancer incidence at 40 weeks between castrated males (50%) and ARKO males (0%) and between DHT-supplemented ARKO males (25%) and ARKO males/females (0%) suggested the involvement of non-androgen-mediated AR pathways and androgen-mediated non-AR pathways, respectively, in inducing bladder carcinogenesis.

Other Animal Models

Using mouse xenograft models, the role of AR signals in bladder carcinogenesis was further investigated. Tumor development of the R198 transplantable line, derived from a human bladder carcinoma expressing the AR, was prevented by castration in 50% of adult male mice (60). Targeting the AR via the expression of AR-siRNA in xenograft tumor cells or administration of anti-AR molecule ASC-J9 that selectively degrades AR protein (61) in mice also resulted in delayed tumor formation (31).

Effects of androgens and AR signals on the expression of enzymes that activate or inactivate carcinogens have also been assessed in non-neoplastic bladder cells. Castration down-regulated cytochrome P450 CYP4B1, which activates amines to more genotoxic substances, in male rat bladders, and testosterone supplement restored CYP4B1 levels (62). We have recently shown that AR signals reduce the levels of UDP-glucuronosyltransferase [UGT (for human)/Ugt (for mouse)], which is known to play an important role in detoxifying bladder carcinogens, such as BBN and NNAL (55). In the SVHUC human normal urothelial cell line stably expressed with AR, DHT treatment down-regulated the expression of UGT subtypes, and flutamide antagonized the DHT effects. Additionally, Ugt levels were higher in mouse bladders from wild-type females than those from wild-type males, those from castrated males than those from intact males and in those from ARKO males than those from wild-type littermates. The findings from these two studies (55,62) suggest that androgen-mediated AR signals promote bladder carcinogenesis by up-regulating CYP4B1 and down-regulating UGTs in the bladder.

AR SIGNALING IN BLADDER TUMOR PROGRESSION

Although it remains controversial whether the AR pathway always plays a dominant role in bladder cancer progression, evidencing the effects of androgens on the growth of AR-positive tumors *in vitro* and *in vivo* are promising.

In Vitro Effects of Androgens-AR

Several *in vitro* analyses have assessed the effects of androgens and/or AR signals on the growth of bladder cancer cells. First, AR-mediated transactivation can be modulated by androgens in bladder cancer cells (30,31,63). Androgens increased AR-responsive reporter gene activity, which was abolished by AR antagonists or AR knockdown via RNA interference technology, indicating the functional activity of their endogenous AR. Second, AR expression can be altered by androgen treatment in bladder cancer cells. Boorjian et al. (33) showed that treatment with 1 nM R1881, a synthetic androgen, for 48 h resulted in a considerable decrease/little change in AR protein expression in UMUC3/TCC-SUP cells, respectively. Our western blot analysis then showed modest increases in endogenous AR expression in both lines, but not in exogenously overexpressed AR in AR-negative 5637 cells, after DHT treatment at 1 nM for 24 h (63). Third, AR signals have been shown to have stimulatory effects on bladder cancer cell growth (31–33,63,64). In cell viability assays, androgens induced AR-positive cell proliferation, and anti-AR compounds (e.g. flutamide, ASC-J9) or silencing of AR eliminated the effect of androgens. AR knockdown in bladder cancer cells was also shown to result in increased apoptosis and decreased migration in the presence or absence of androgens (64). Finally, as shown in prostate cancer cells, EGF, in conjunction with androgen, augmented AR transcriptional activity and protein expression as well as cell proliferation in bladder cancer lines, suggesting the EGF effects through activation of the AR pathway (65). Taken together, *in vitro* evidence strongly supports the stimulatory role of AR signaling in bladder cancer progression.

In Vivo Effects of Androgens-AR

In an earlier study using R198-bearing male mice, the tumors grew more rapidly following administration of DHT than those in untreated controls (60). Subsequently, using mouse xenograft models for AR-positive bladder cancer, others and we have shown that androgen deprivation via castration and/or flutamide or ASC-J9 treatment (31) as well as AR silencing via electroporation to deliver AR-siRNA (64) significantly reduces tumor size. Coincidentally, decreased cell proliferation index and increased apoptotic index, as well as decreases in the expression of angiogenesis/metastasis-related factors, including basic fibroblast growth factor, vascular endothelial growth factor and matrix metalloproteinase (MMP)-9, were observed in these xenograft tumors where the AR was targeted (31,64). Inhibitory effects of androgen ablation on tumor progression were confirmed further in the UPII-SV40T transgenic mouse model that expresses the SV40 large T antigen particularly in the urothelium and spontaneously develops bladder cancer (32). Castration retarded tumor growth and increased the expression of thrombospondin-1 (TSP1), which inhibits angiogenesis, while DHT supplement restored the effects of androgen ablation on tumor size and TSP1. Thus, *in vivo* studies have attempted to determine the clinical relevance of *in vitro* findings and to address the feasibility of future therapeutic application.

Most recently, anti-tumor effects of the bladder-cancer-specific adenovirus carrying E1A-AR were assessed both *in vitro* and *in vivo* (66). Infection of the viruses targeting AR-positive bladder cancer led to an inhibition of cell proliferation and a regression of implanted tumors.

AR-Regulated Molecules in Bladder Cancer Cells

Through the aforementioned *in vitro* studies, it has been shown that androgens are able to modulate the expression/activity of various molecules via the AR pathway in bladder cancer cells. These molecules related to cell proliferation, tumor growth and/or metastasis are summarized in Table 2.

Activation of the EGF receptor (EGFR) family, such as EGFR and ERBB2, is known to involve tumorigenesis and tumor progression of a variety of malignancies, including bladder cancer. Consequently, the efficacy of targeted

Table 2. Reported effects of androgens/AR signals on the expression/activity of key molecules related to tumor growth in bladder cancer cells

Molecules	Main function	Androgen effect	Reference
MMP-9	Cell proliferation, migration and invasion	Up-regulation	64
Caspase-3/7	Apoptosis executor	Down-regulation	64
Cyclin D1	Cell cycle regulator	Up-regulation	64
Bcl-xL	Anti-apoptotic factor	Up-regulation	64
NEP	Cell surface metalloprotease	Up-regulation	34
EGFR	Cell proliferation, migration and invasion	Up-regulation	63
ERBB2	Cell proliferation, migration and invasion	Up-regulation	63
AKT	Cell survival	Up-regulation	63
ERK1/2	Cell survival	Up-regulation	63
IL-6	Cytokine	Down-regulation	30
β-Catenin	Cell growth and adhesion	UP-regulation	69

MMP, matrix metalloproteinase; NEP, neutral endopeptidase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; IL, interleukin.

therapy directed at EGFR signals has been assessed in bladder cancer (67). Recently, we demonstrated that androgen up-regulated the expression of EGFR and ERBB2 as well as the levels of phosphorylation of their downstream proteins AKT and extracellular signal-regulated kinase1/2 via the AR pathway in bladder cancer cells (63). Together with our other recent findings showing EGF-induced cell proliferation via modulating AR signals (65), cross-talk between AR and EGFR pathways was suggested to play an important role in bladder cancer progression.

Intravesical administration of bacillus Calmette-Guerin (BCG) is so far the most effective form of adjuvant therapy for high-risk superficial bladder cancer. Among cytokines elicited in response to BCG, interleukin (IL)-6 likely contributes to promotion of BCG adherence to bladder cancer cells and consequently to determination of BCG treatment efficacy. In AR-positive bladder cancer lines, DHT down-regulated BCG-induced IL-6 expression, and antiandrogens reversed the DHT effects (30). These results suggest that pharmacological manipulation of AR-mediated suppression of IL-6 has therapeutic value during intravesical BCG treatment.

Activation of Wnt/β-catenin signaling has been reported to correlate with poor prognosis in patients with bladder cancer (68). In our bladder TMAs, co-expression of nuclear AR and β-catenin was associated with tumor progression. In AR-positive bladder cancer lines, we further showed that DHT increased the expression of an active form of β-catenin and enhanced its nuclear translocation (69). Thus, it appeared that androgen was able to activate β-catenin signaling via the AR pathway in bladder cancer cells.

CONCLUSIONS

Given the current understanding of a critical role for AR signaling, as well as the involvement of other nuclear hormone receptor signals (70), in bladder tumorigenesis and tumor progression, bladder cancer should be accepted as a member of endocrine-related neoplasms. Although the underlying mechanisms of how AR signals regulate bladder cancer growth remain far from fully understood, the available data strongly support that targeting the AR provides effective chemopreventive and therapeutic approaches for urothelial carcinoma. Indeed, a variety of therapeutic options are available for AR-dependent prostate cancer (12), and most of these anti-AR therapies may be able to be applied to bladder cancer. Their preventive roles appear to be more convincing because the AR pathway is likely essential for bladder cancer initiation, while there are no clinical data showing that androgen deprivation therapy in prostate cancer patients reduces the incidence of subsequent bladder cancer. In contrast, the usefulness of anti-AR therapy may be limited, for example, for patients with bladder cancer possessing a functionally active AR. Further understanding of the roles of AR as well as other molecules directly or indirectly regulated by androgens may help to develop better strategies for the management of bladder cancer.

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Conflict of interest statement

None declared.

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