Clinical and therapeutic implications of sex steroid hormone receptor status in urothelial bladder cancer

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ABSTRACT

Studies on the clinical profile of urothelial bladder cancer (UBC) have shown significant gender differences, namely, higher occurrence in males (male-to-female ratio of 3.5:1) and an advanced stage of disease at the time of diagnosis with rapid progression of the disease after initial diagnosis seen more commonly in females. The relationship between gender and UBC is complex and probably influenced by biological and epidemiological factors. Potential contributory factors such as sex steroid hormone pathway, gender difference in environmental carcinogen exposure, metabolic enzyme activity, and disparities in the intensity of diagnostic evaluation could probably explain the demographic trends in UBC. This comprehensive review of Medline publications during the period 2009–2019 attempts to identify the possible role of sex hormone receptors in gender variation and sexual dimorphism in the occurrence and progression of UBC. The clinical implications of identifying sex steroid receptors on factors such as disease prognostication and the therapeutic role of anti-androgens in the prevention and progression of UBC are critically reviewed. There is now significant evidence in literature to suggest the possible role of sex steroid hormone receptor-mediated signals in the genesis and progression of UBC. These receptors include androgen receptors, estrogen receptors, progesterone receptors, and various other orphan receptors. Excessive or reduced expression of these receptors, as well as alterations in their upstream or downstream pathways, correlate well with the clinical and therapeutic outcomes of UBC.

INTRODUCTION

Bladder cancer (BC) is the fourth most commonly diagnosed cancer in males and eleventh most common cancer in females. Nearly 95% of BC is transitional cell carcinoma or urothelial bladder cancer (UBC). Studies on the clinical profile of urothelial bladder cancer (UBC) have shown significant gender differences, namely, higher incidence in males (male-to-female ratio of 3.5:1) and an advanced stage of disease at the time of diagnosis with rapid progression of the disease after initial diagnosis seen more commonly in females. Potential contributing factors such as sex steroid hormone pathway, gender difference in environmental carcinogen exposure, metabolic enzyme activity, and disparities in the intensity of diagnostic evaluation could probably explain the demographic trends in UBC. It is imperative for clinicians to identify the possible role of sex hormone receptors in this gender variation and sexual dimorphism in the occurrence and progression of UBC, which will allow optimization of the treatment of the disease and improvement in the quality of care in both genders. We comprehensively review Medline® publications on the role of sex hormone receptors in UBC, published during the period 2009–2019.

DOES SEXUAL DIMORPHISM EXIST IN UROTHELIAL BLADDER CANCER?

UBC shows a significantly higher incidence in males, in the ratio 3.5:1, compared to females. The incidence was even more alarmingly high in an Indian population,

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with a male-to-female ratio of 8.6:1.[1] Women are more likely to be diagnosed with muscle invasive UBC than men (85.2% and 50.7%, respectively), and women with UBC are approximately twice more likely than males to die from this disease.[2-5] Further, even when men and women smoked at comparatively high levels, the risk of UBC among women was 30%–50% higher than men, and there was a delay in diagnosis in women with hematuria.[6,7] The response of intravesical therapy for nonmuscle invasive UBC was also poor in females. A relatively lower rate of utilization of chemotherapy (45% vs. 52% in males) in women has been reported to be responsible for the survival gap.[8]

Wang *et al.* reported that there were significant racial differences in patient characteristics in carcinoma bladder, including gender, marital status, age at diagnosis, treatment strategies, grade, and stage and survival time in various regions of the USA.[9] Interestingly, Henning *et al.* reported that there was no gender-related differences in clinical symptoms though women were more likely to be treated for voiding complaints or suspected urinary tract infection without further evaluation by a clinician.[10]

In a study on 259 patients undergoing radical cystectomy (RC), it was observed that gender did not independently predict recurrence-free survival (RFS), cancer-specific survival , or overall survival (OS) on a multivariate analysis.[11] However, women had greater propensity for extravesical ($\geq pT_3$) disease (53.2% vs. 33.9%, $P = 0.03$) and heterotopic urinary diversion (72.3% vs. 49.5%, $P = 0.006$). However Wang *et al.* reported high mortality-to-incidence ratio in females for UBC being attributable to disparities in health-care delivery systems.[12]

Assessment of US vital rates and survival data from the Surveillance, Epidemiology, and End Results (SEERs) database for cancers by sex and age for the period 1977–2006 showed that male-to-female mortality rate ratio was higher among males in various cancers including UBC, though cancer survival disparities were not so pronounced.[13] This suggested that sex-related cancer disparities were more strongly related to etiology than prognosis.

In a Norwegian study, it was observed that mortality risk was significantly higher for women than men up to 2 years after UBC diagnosis, especially for muscle invasive cancer.[14] Thereafter, risk rates appeared to be higher in men. This difference was probably attributable to adverse T stage distribution in women at the time of treatment. Kluth *et al.* in a retrospective study of patients with primary T_1High-Grade BC across seven tertiary care centers who underwent transurethral resection of bladder with or without intravesical instillation reported that female gender was associated with a higher risk of disease recurrence.[15] However, there was no difference between genders with

regard to disease progression, cancer-specific mortality, and any-cause mortality.

A study on the data among women in the US Nurses' Health Study cohort found that postmenopausal women were at increased risk of developing UBC.[16] Early age at menopause (≤45 years) was associated with a statistically significant increased risk of UBC. However, there was no significant association of age at menarche, age at first child birth, or use of exogenous hormones with UBC risk.

These studies show that the relationship between gender and UBC is complex and probably influenced by a variety of biological and epidemiological factors.

WHAT ARE THE POSSIBLE FACTORS RESPONSIBLE FOR THE SEXUAL DIMORPHISM AND THEIR CLINICAL IMPLICATIONS IN UBC?

The gender-specific differences in the outcomes of various malignancies seem to be multifactorial and include genetic, physiological, and anatomical characteristics; varying exposure and responses to carcinogens; and treatment-related peculiarities.[17]

Anatomical factors
Most of the retrospective randomized controlled series suggested that female gender was associated with poor outcomes,[7,10,18-26] whereas other studies found no differences in the outcome of treatment of UBC among both genders in pT_{4a} UBC.[7,10,15,18-33] Several multicentric studies and SEER analysis showed that females seem to experience poorer survival rates compared to men with similar stage of disease after RC, probably due to the infiltration of vagina or uterus which was not pronounced in metastatic UBC who underwent first-line cisplatin-based chemotherapy.[34-37]

Elderly men typically have a thicker detrusor muscle compared to women due to the high prevalence of BPH causing bladder outlet obstruction, which delays the extravesical spread of tumor in males.[38] In addition, the common embryonic development of trigone, bladder neck, and upper part of vagina could be a contributing factor to the more invasive pattern of UBC in females.[39] Denonvillier's fascia, prostate, and the prostatic urethra impair the early lympho-vascular extension of the tumor in males.[40-42]

Clinical factors
It has been observed that the interval from the onset of hematuria to the diagnosis of UBC was longer in women than in men.[7,24] Moreover, females with hematuria were more likely to be diagnosed with urinary tract infection.[10,23] A delay in the diagnosis and treatment of UBC was obviously associated with more advanced disease stage at diagnosis in females.

Women seemed to receive more frequently intra-vesical BCG immunotherapy compared to males prior to RC, probably due to apprehension among the clinicians in administering BCG in men due to the potential risk of prostatitis.[43] Rink *et al.* reported that women were often elder than men at the time of RC, leading to an increased risk of cancer-specific mortality.[44] In addition, female patients received incontinent urinary diversion more popularly compared to men.[45]

Although TURBT in females additionally increased the risk for intraoperative bladder perforation due to the thinner detrusor muscle,[38,46] unintentional, yet significant discrepancies were observed in the peri-operative quality of care in men and women, leading to higher mortality and complications in female patients during the first 3 months after RC.[26,47,48] Women also had higher intraoperative blood loss requiring peri-operative blood transfusion, which had a negative impact on the survival of UBC patients undergoing RC.[25,26]

Carcinogenic factors

Cigarette smoking has been implicated as the most relevant risk factor for the development of UBC, increasing the incidence by 2–6 fold, independent of gender.[49,50] Smoking has a dose-dependent negative impact on survival in UBC undergoing treatment due to hyper-methylation of tumor suppressor genes causing unfavorable outcomes.[50-53] Women with a history of tobacco use had also an increased risk of disease progression.[54] Although smoking prevalence has been declining worldwide among both genders, tobacco use in women has been found to be rising in females compared to men, potentially contributing to gender-specific UBC incidence and survival.[55] A large population-based, case–control study also found that women using permanent hair dyes were at an increased risk of developing UBC, especially those with N acetyl transferase (NAT-2 slow acetylation phenotype).[56] An increased risk of UBC development has been found in patients with a history of gonorrhea and human papillomavirus infection, which are more common in females.[57,58]

It is possible that there could be gender-specific differences in the degradation of carcinogens at the molecular level.[59] Gender variations in hydroxylation, acetylation, and glucuronidation pathways including various enzymes such as uridine-diphosphoglucoronosyl transferase (UGT) and NAT-2 play an essential role in the degradation of aromatic amines, contributing to carcinogenesis. Glutathione-S-transferase M_1 ($GSTM_1$) is an enzyme that degrades certain carcinogens by conjugation to glutathione.[59,60] It is worthwhile studying whether there are gender differences in UGT expression, NAT-2 status, and $GSTM_1$ expression, contributing to the sex-specific differences in UBC susceptibility.

The various nonsex steroid receptor factors which contribute to early disease progression and recurrence in females as compared to males are summarized in Table 1.

Sex hormonal factors

McGrath *et al.* had reported that postmenopausal women were at a higher risk for developing UBC compared to premenopausal women.[16] However, an older age at menarche, parity, and combined hormone replacement therapy with estrogen and progesterone seemed to reduce the risk of UBC.[2,18,61] Conversely, the supposed protective effect of postmenopausal hormone replacement with estrogen and progesterone did not give protection against the risk in treatment periods lasting for more than 10 years.

The androgen receptor (AR) is a steroid hormone receptor activated by the androgens, testosterone, and dihydrotestosterone.[62] It has been found that in UBC, the AR expression decreased with increasing pathological stage, with 88.9% of pTaUBC and 0% of pT_3UBC expressing the AR.[21] In addition, co-regulators of the AR, enabling the formation of the AR transcriptional complex, have been found to be expressed in 85%–100% of UBC specimens.[63] Additionally, high-risk UBC might lose the expression of 5α reductase, leading to decreased conversion of testosterone to the more potent DHT.[2] The molecular mechanisms by which androgens promote the development and progression of UBC include the increased androgen-dependent susceptibility of the urothelium to carcinogens, impaired degradation of carcinogens by androgen-dependent pathways, or direct oncogenic effects of androgens.[20]

Some studies have reported that ARs could influence various other signaling pathways by interacting with β-catenin, cyclin-d, and estimated GFR to promote the carcinogenesis of aggressive biologic behavior.[62,64-66] Nam *et al.* in a retrospective study on tumor specimens from patients treated for UBC observed that recurrence was slow when the AR was expressed in the specimens, and it could be a predicting factor for stage, number of tumors, carcinoma *in situ*, and recurrence.[67] A review of 12 relevant studies with 1652 patient samples for AR expression concluded that there was a positive correlation of AR expression low

Table 1: Factors other than sex steroid receptor status contributing to early progression/recurrence of urothelial bladder cancer in female gender

Thinner detrusor layer compared to males
Early infiltration of adjacent organs
Delay in diagnosis
Delay in starting treatment
Poorer quality of health-care delivery system
Increased incidence of smoking
Increased incidence of UTI
Differences in the degradation of carcinogens at molecular level
Changes in hormonal *milieu* in postmenopausal age group

UTI=Urinary tract infection

tumor grade, low tumor stage, and low recurrence rate in Caucasian patients.[26]

The expression of estrogen receptor β (ERβ) has been found to be increasing with advancing pathological tumor stage and higher grading.[22] Nearly 53% of pTaUBC and 75% pT$_4$ tumor as well as 58% of WHO Grade 1 and 2 tumors and 70% of Grade 3 tumors were seen to express ERβ, respectively. In contrast, the ERα was rarely expressed in the urothelium and not associated with UBC showing progressive behaviors.[19,22] The role of progesterone receptor A in UBC which was expressed in the squamous epithelium of the urethra has not been completely understood.[3] *In vitro* and animal experiments have suggested the usefulness of tamoxifen, an anti-estrogen in reducing UBC incidence following carcinogen exposure.[68]

In a meta-analysis of 2049 patients from 13 retrospective studies, the difference in ERα expression between nontumors and tumors was significant, while those of AR or ERβ was not statistically significant.[69] AR positivity in tumor strongly correlated with gender (male vs. female; odds ratio [OR] = 0.658; P = 0.027) or tumor grade (low grade vs. high grade; OR = 0.575; P < 0.001). ERβ-positive rates were significantly higher in high-grade and muscle invasive tumors.

Survival analysis revealed an association between AR expression and better RFS as well as between ERβ expression and worse recurrence-free or progression-free survivals in patients with nonmuscle invasive UBC.[70] These results suggest downregulation of ERα expression in bladder cancer compared with nonneoplastic urothelial tumors. Patients with upper urinary tract urothelial tumors, positive for either ERα or PR, had a significantly higher risk of disease-specific mortality compared to those which were negative for both.[71] The various sex hormonal factors contributing to the gender differences in UBC and progression are summarized in Table 2.

Genetic factors

Various studies have shown that single-nucleotide polymorphisms on chromosome 8q24, especially PSCA gene, could be associated with an increased risk of UBC. In its promoter region, the PSCA gene contains an androgen response element. The lower androgen level in women could cause an easy loss of AR reactivity, leading to more aggressive tumor biology in female UBC.[2,20]

WHAT ARE THE THERAPEUTIC IMPLICATIONS OF SEX STEROID HORMONE DEPENDENCY OF UBC?

Gil *et al.* observed that dihydroxy testosterone (DHT) treatment significantly increased AR expression in bladder cell line HCV29 and also activation of Akt/GSK-3 β signaling pathway, which play a center role in cancer progression in

Table 2: Role of sex steroid receptor status contributing to urothelial bladder cancer in both genders

AR status (biphasic effect)
Promotes urothelial bladder carcinogesis by
Increasing androgen dependent susceptibility of urothelium to carcinogens
Impairing degradation of carcinogens
Directly affecting oncogenesis
Activating other signaling pathways of carcinogenesis
Decreases progression of urothelial bladder cancer by
Decreasing the overall expression of AR
Increasing the co-regulators of AR
Decreasing the expression of 5-ARIs
ER status
ERα inhibits UBC
ER β promotes UBC
PR status
Role is yet to be understood

UBC = Urothelial bladder cancer, AR = Androgen Receptors, ER = Estrogen receptors, PR = Progesterone receptor, ARIs = Alpha reductase inhibitors

in vitro studies.[72] In a review of 196,914 patients diagnosed with histologically confirmed localized prostate cancer during the period 2000–2009, identified in the SEER Medicare Insurance Program Linked Database, it was observed that 2495 (1.3%) of these patients developed UBC during follow-up.[73] After stratification to androgen deprivation therapy (ADT0, the 10-year cumulative incidence was 1.75% in the ADT untreated group, and the 10-year cumulative incidence rate was 1.99%. In a retrospective review of 20,328 patients with prostate cancer diagnosed during 1991–2013, 239 (1.2%) men were identified to have primary UBC.[74] With a median follow-up of 62 months, 38 (50%) patients without ADT experienced UBC recurrence, while 19 (22%) did in ADT group.

Kawahara *et al.* in *in vitro* studies demonstrated that culture with enzalutamide (P = 0.028), hydroxy flutamide (P = 0.033), or bicalutamide (P = 0.038) resulted in prevention/retardation of tumor formation.[75] Enzalutamide has also been shown to inhibit growth of AR-expressing bladder cancer cells with both acquired and intrinsic gemcitabine-resistance through cell cycle arrest.[76]

Autophagy has been postulated as a potential mechanism underlying ENZ-resistant bladder cancer. Blockade of autophagy using chloroquine, 3-methyladenine, and bafilomycin A1 on J82, T24, and UMUC3 bladder cancer cell lines showed significantly increased ENZ-induced apoptosis.[77] Thus, concurrent treatment with autophagy inhibitors and enzalutamide could be a novel therapeutic strategy for bladder cancer.

There is an observational study on Prostate, Lung, Colorectal, Ovarian Carcinoma trial data, in 72,370 men with more than 13-year study follow-up, of which 8.4% had reported the use of finasteride.[78] UBC was diagnosed in 1.07% (65/6069) of those who reported finasteride compared to 1.46% (966/66,301) of those who reported no use of the

drug. There are two reports on the potential therapeutic role of tamoxifen in advanced UBC. One report suggested that the regression of metastatic urothelial cancer disease could have occurred because of the initiation of tamoxifen-induced gynecomazia.[79] In the other study using high dose of tamoxifen, in addition to systemic chemotherapy with cisplatin, methotrexate, and vinblastine, there was better overall response rate of 58% compared to historical conventional cisplatin-based combinations.[80]

Shang *et al.* observed that the combination of BCG and the anti-estrogen ICI 182780 led to better suppression of UBC than BCG alone in *in vitro* and *in vivo* studies.[81] The mechanisms of action proposed include the potentiation of BCG attachment/internalization to the UBC cells by anti-estrogens and increased monocyte/macrophage migration toward UBC cells, leading to more tumor necrosis factor alpha (TNF-α) junction to kill the cells and countering the estrogenic downregulation of expression of interleukin-6, integrin-α5 β1, and TNFα (which are key molecules to mediate BCG-induced monocyte/macrophage recruitment and kill UBC cells).

There is an interesting study to assess whether receipt of 5-alpha reductase inhibitors (5-ARIs) influenced the findings on surgical pathology at the time of RC and subsequent clinical outcomes.[82] The study cohort included 338 men; 48 patients (14%) were receiving dutasteride or finasteride at the time of RC, 58 (17%) metformin, and 195 (58%) statins. Among patients receiving 5-ARIs, there was a lower proportion of positive margins ($P = 0.08$) and lymphovascular invasion ($P = 0.05$). With better OS, no similar tendencies were observed with metformin or statins.

WHAT ARE THE PROPHYLACTIC IMPLICATIONS OF SEX STEROID HORMONE DEPENDENCY OF UBC?

In a Japanese study on 53 TURBT specimens performed for non muscle invasive BC (NMIBC), quantification of AR in RNA expression using real-time quantitative polymerase chain reaction was done and correlated with NMIBC recurrence.[83] It was concluded that high AR in RNA-expressing group tended to have a longer median RFS than in low AR group. Multivariate analysis showed female sex tumor size ≥ 3 cm and low AR in RNA expression to be independent predictors of shorter RFS. Kaneko and Li showed that the loss of X-linked lysine demethylase 6A (KDM 6A), a sexually dimorphic gene, increases UBC risk in female mice and mutations or reduced expression of human KDM 6A predicted poor prognosis of female UBC patients.[84] Trilla-Fuertes *et al.* observed that muscle invasive tumors with predominantly basal cells were highly proliferative, making them good candidates for neoadjuvant chemotherapy.[85] The luminal cell tumors were good candidates for therapy with AR inhibition.

CONCLUSIONS

There is now adequate evidence in literature to suggest the possible role of sex steroid hormone receptor-mediated signals in the genesis and progression of UBC. There is excessive or reduced expression of these receptors, and alterations in their upstream or downstream pathways correlate well with the outcomes of UBC. AR activation correlates with the promotion of urothelial tumorigenesis and progression. ERα expression is protective against the development of urothelial cancer, whereas ERβ expression is up regulated in high-grade and/or muscle invasive UBC. There exists an inverse relationship between AR expression and advanced disease, indicating the biphasic nature of receptor behavior during the natural course of the disease. These observations support the theory that UBC is a member of hormone responsive tumors, regulated by sex steroid receptor signaling pathways. However, the underlying mechanisms of how AR, ER, and related signals regulate the UBC growth need further elucidation.

The lower incidence of UBC in patients undergoing anti-androgen therapy for prostate cancer opens up new vistas in the prophylactic and therapeutic management of UBC in patients who are at high risk for contracting the disease. Immunohistochemistry of surgical specimens for AR and ER could probably serve as a tool for prognostication of the disease. The difficult availability of AR and ER assays and the cost of tests would be limiting factors for extrapolating the research test results to clinical applications immediately. More studies are also necessary in a country like India, where the cancer etiology, biology, behavior, and treatment vastly vary across the community.

Future studies will probably focus on testing whether ERα agonist, 4,4',4"-(4-propyl-1[H]-pyrazole-1,3,5-tiyl) triphenol or the ERβ antagonist, 4-(2-phenyl-5,7-bis [trifluro methyl] pyrzolo [1,5-α] pyrimidin-3-yl) phenol, can inhibit genesis or progression of UBC.[86,87] Also, the therapeutic role of employing antibodies to chemokines which normally get downregulated by ERα for the suppression of oncogenesis needs critical appraisal.[88] Availability of similar drugs in future perhaps will help us achieve the ultimate goal of preventing UBC in the susceptible subset of patients.

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