Original Article

Clinical study of steroid receptors in nonmuscle invasive bladder cancer: A domain worth revisiting

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ABSTRACT

Introduction: The prognostic significance of steroid receptors in bladder cancer remains controversial. This study was designed to determine the expression status of androgen receptor (AR), estrogen receptors (ERα and Erβ), and its potential role in predicting survival in patients with nonmuscle invasive bladder cancer (NMIBC).

Methods: Sixty patients of NMIBC were screened and 57 (41 males and 16 females) were included in our study. The tissue microarray slides were evaluated by pathologists blinded to the clinical information. Association of distribution of steroid receptors with stage, grade, progression, and recurrence was seen.

Results: The mean age of the population was 60.9 ± 9.3 years. Pathologically, majority of the patients were Ta (Ta: T1 stage 61.4% vs. 38.6%). Nine (15.8%) of the tumors stained positive for AR while one (1.8%) tumor stained positive for ERα and 36 (63.2%) tumors stained for ERβ. A higher proportion of male NMIBC stained positive for AR (19.5% vs. 6.2%, $P = 0.420$) while ERβ positivity was higher in females (58.5% vs. and 75%,$P = 0.247$). AR-negative tumors showed higher recurrence (20/48%–42%) as compared to AR-positive tumors (2/9%–22%). ERβ-positive tumors showed higher recurrence (15/36%–42% vs. 7/21%–33%, $P = 0.179$). Progression-free survival (PFS) was found to be significantly lower for ERβ-negative group (log-rank test $P = 0.035$).

Conclusion: AR and ERβ positivity is found in NMIBC patients while ERα shows minimal staining in NMIBC patients. Although it did not reach a statistical significance, a higher proportion of AR-negative and ERβ-positive tumors recurred as compared to AR-positive and ERβ-negative patients. PFS was significantly lower in ERβ-negative group. Further exploratory studies on larger sample sizes are required to validate these findings in NMIBC patients.

INTRODUCTION

Bladder cancer, which is mostly urothelial carcinoma, is one of the most frequently diagnosed neoplasms with different gender distribution.[1] Although men are nearly 3–4 times more likely to develop bladder cancer than women in the Western population and nine times in the Indian subpopulation, women present with more advanced disease and have poorer survival. Apart from the difference in the status of smoking between the two genders, hormonal milieu has been incriminated affecting the incidence.[2,3]

These observations have prompted investigations of steroid hormones and their receptor signals, especially androgens/estrogen receptors (AR/ERs) in bladder cancer, which have demonstrated their critical roles in tumorigenesis and tumor progression.[4,5]

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Conflicting data were published regarding the expression of sex hormone receptors in urothelial tumors of the lower urinary tract, and its associations with tumor grade/stage or patient outcomes have been reported.[4] Furthermore, there has been no data on the Indian subpopulation that has a starkly different demographic distribution. Therefore, in the current study, we aimed to determine the expression status of AR, ERα, and ERβ immunohistochemically detected in patients with nonmuscle invasive bladder cancer (NMIBC) and its potential role in the recurrence and progression of urothelial carcinoma.

MATERIALS AND METHODS

The study was an ambispective observational study, carried out in the Department of Urology, PGIMER in collaboration with the Department of Histopathology, PGIMER, Chandigarh. Ethics committee (No: INT/IEC/2020/SPL-1474) approval was obtained. The clinical details of patients with urinary bladder mass who underwent transurethral resection of bladder tumor (TURBT) at our center from July 2016 to June 2021 were noted. Patients of NMIBC (Ta, Tis, T1) with complete treatment details and whose tissue blocks were available for tissue microarray (TMA) construction were included in the study. Patients of papillary urothelial neoplasm of low malignant potential, muscle invasive bladder cancer (MIBC), nonurothelial carcinoma (variant histology) as squamous-cell carcinoma, adenocarcinoma, small cell carcinoma, etc., and patients who had upstaging to MIBC after restage TURBT were excluded from the study. Informed signed consent was taken from all patients included in the study.

Sixty patients consisting of 42 males and 18 females were included. There was loss of tissue during staining for three patients. Hence, a total of 57 patients were included in the final analysis. The tumor was classified according to the American Joint Committee on Cancer/International Union Cancer Consortium 2017 Tumor, Nodes, and Metastases Staging Classification[6] and graded according to the World Health Organization Classification of Tumors of the Urinary System and Male Genital Organs 2016.[7] The NMIBC cases were stratified into three risk groups for further guiding the therapeutic decisions based on the European Association of Urology risk assessment of NMIBC.[8]

Recurrence was defined as a tumor of a lower or similar stage/grade appearing during any point of the treatment course and progression was defined as tumor of a higher stage/grade appearing during any point of the treatment course. The follow-up data including recurrence and progression were noted based on check cystoscopy findings and repeat TURBT if needed. Association with the steroid receptor distribution with stage, grade, progression, and recurrence was analyzed.

A 2–3 μ thick section of TMA-embedded paraffin block was used to carry immunohistochemistry (IHC) staining [Figure 1]. AR receptor was stained using Cell Marque, SP107 clone with dilution 1:100, EPα with Cell Marque, SP1 clone with dilution 1:100 and EPβ with Novus Bio, 14C8 clone with 1:50 dilution. The TMA slides were evaluated by pathologists who were blinded to the clinical and pathological information. AR and ERβ staining was detected as brownish nuclear staining and all these stains were manually scored by the German immunoreactive score.[9]

Analysis was carried out using IBM-S tatistical Package for Social Sciences (IBM Corp., Armonk, N.Y., USA) for Windows version 21.0. The normality of continuous variables was initially checked using Kolmogorov–Smirnov and Shapiro tests for normality. Normally distributed data were expressed as mean with standard deviation (SD) and statistical significance was checked using an independent sample *t*-test. Nonparametric data were expressed as median with range and statistical significance checked using Wilcoxon rank-sum test or Mann–Whitney test wherever applicable. For analysis of two or more variables for continuous data, analysis of variance and Kruskal–Wallis test were used for parametric and nonparametric data, respectively. Pearson's Chi-square and Pearson's *R* tests were used to analyze the significance of the correlation between the expression of the putative steroid receptors (AR, ERα and ERβ) with that of clinical pathologic variables. Survival analysis was done using the Kaplan–Meier survival curve and log-rank test. A $P < 0.05$ was considered statistically significant.

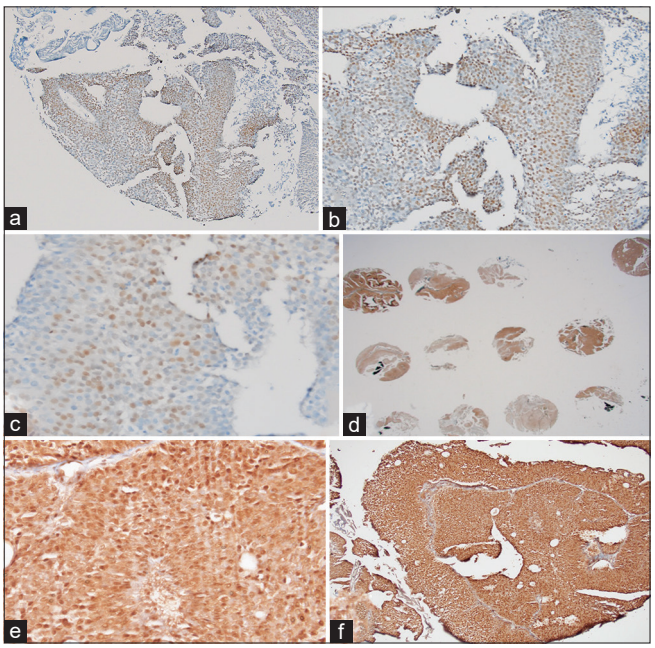

Figure 1: Tissue microarray showing immunohistochemistry staining of low-grade urothelial carcinoma showing nuclear positivity of androgen receptor of weak intensity. Magnification in (a) ×100, (b) ×200, (c) ERα of mild intensity (×200), (d) ERβ of moderate intensity, (e) ×400, (f) ×100

RESULTS

Fifty-seven patients (41 males and 16 females) with NMIBC were included in the study. The mean age of the patients in our study was 60.9 ± 9.3 years. Twenty-one (36.8%) patients were smokers and most of them were male (18).

Most of the patients had a single tumor (82.5%) and most of the tumors were fused papillary (61.4%) while only 3/57 (5.3%) tumors were solid sessile. The histopathology stage of 35 patients (61.4%) was Ta while the stage of 22 patients (38.6%) was T1 [Table 1]. A higher proportion (63.4%) of males presented with Ta stage while among females a higher proportion (43.8% vs. 36.6%) presented with T1 stage ($P = 0.618$). Majority of the tumors were low grade (LG) (71.9%) while only 16 (28.1%) tumors were high grade (HG). Between both genders, a higher proportion of females (37.5%) presented with HG tumor compared to males (24.4%) ($P = 0.343$). Twenty-two patients (38.6%) received intravesical therapy which is reflective of the T1 burden of the disease in the cohort. Most of the patients (86.4%) received Bacillus Calmette–Guérin (BCG) as an intravesical drug while only three patients (13.6%) received a combination of intravesical gemcitabine + docetaxel due to BCG shortage during that period.

Six (10.5%) of the patients had tumor progression. The median (interquartile range [IQR]) time to progression was 43 months (24–50.75) and ranged from 6 to 56 months. Among patients who underwent progression, three patients (50%) progressed to T2 HG, two patients (33.3%) progressed to T1 HG and one patient (16.7%) progressed to T1 LG. Among 41 males, recurrence was found in 17 patients (41.5%), while among females, it was found in five patients (31.2%) ($P = 0.477$). Among males and females who had recurrence, median time to recurrence was lower in females (13 months vs. 17 months, $P = 0.388$). Progression was seen in a higher proportion of females (18.8%) than males (7.3%) ($P = 0.355$). Mean time to progression (SD) among females was lower at 25.67 months (23.03) than males at 47.33 months (10.26) ($P = 0.241$). Of the patients who had multiple lesions in bladder ($n = 10$), recurrence was seen in 50%. Although recurrence was higher when compared to patients who had a single lesion in bladder (36%), it did not reach a significant value ($P = 0.41$). Similarly, the progression of disease was more in patients with multiple tumors in comparison to single lesions (20% vs. 8.5%) but was not of significant value ($P = 0.28$). In our study, 22 patients received intravesical therapy. Six patients (27.3%) among them showed recurrence while in patients who did not receive any form of intravesical therapy 16 (45.7%) showed recurrence. A higher proportion of patients who did not receive intravesical therapy showed recurrence ($P = 0.164$). Among patients who received intravesical therapy, two (9.1%) patients showed progression while patients who did not receive any form of intravesical therapy four (11.4%) showed progression. A subgroup analysis was done to rule out intravesical therapy as a confounding factor in assessing recurrence and progression in our study. Out of the 22 patients who received intravesical therapy, recurrence was seen in 6 (27.2%) patients while progression was seen in 2 (9%) patients. In the 35 patients, who were not given any form of intravesical therapy, recurrence and progression of disease was seen in 16 (45%) and 4 (11.4%) patients, respectively. No significant difference ($P = 0.163$) was found in the recurrence of disease between patients who received intravesical therapy when compared to those who did not receive it. Similarly, there was no significant difference in the progression of disease ($P = 0.77$) between the two groups. The effect of receptor status on progression-free survival (PFS) was also insignificant [Supplementary Figures 1 and 2] in the two groups (log-rank test $P > 0.05$).

Table 1: Clinicopathologic characteristics of patients included in the study sample

Characteristics	*n*
Male: female	41:16
Mean age (years)	60.9±9.3
Mean follow up duration (months)	18 (9–44)
Smoker	21
EAU risk group	
Low	19
Intermediate	15
High	23
Pathologic stage and grade	
Low grade pTa	35
Low grade pT1	6
High grade pT1	16
Intravesical therapy given	22
Recurrence	22
Time to recurrence (months)	15.50 (7–24.75)
Progression	6
Time to progression (months)	43 (24–50.75)

EAU = European association of urology

On IHC staining, nine (15.8%) of the tumors stained positive for AR with staining Grade 1 + for six (10.5%) tumors and 2 + for three (5.3%) tumors. Only one (1.8%) of the tumors stained positive for ERα with 1 + grade. Thirty-six (63.2%) of the tumors stained positive for ERβ with Grade 1+, 2 + and 3 + for 12 (21.1%) tumors each.

Supplementary Table 1 shows stage-wise and grade-wise distribution of AR receptors. Among four AR-positive Ta LG tumors 2 (5.7%), all showed Grade 1 + and 2 + staining. Two (33.3%) T1 LG tumors were stained with Grade 1 + while among three T1 HG tumors two (12.5%) were stained with Grade 1 + and one (6.2%) stained with Grade 2+. Among all tumor samples, only one (1/57) tumor of T1 HG stained positive for ERα. Thus, its association with other variables was not analyzed.

Among 35 Ta LG tumors, 21 tumors (60%) stained positive for ERβ, while among T1 LG tumors, five (83.3%) stained positive, and in T1 HG tumors 10 (62.5%) stained positive ($P = 0.655$). Among 21 ERβ-positive Ta LG tumors six (17.1%) stained with Grade 1+, seven (20%) with Grade 2 + and eight (22.9%) with Grade 3+. Three (50%) T1 LG tumors stained with Grade 2 + and two (33.3%) stained with Grade 3+. Among T1 HG tumors, six (37.5%) stained with Grade 1+, two each (12.5%) stained with Grade 2 + and 3+.

Among nine patients with AR-positive tumor, two (22.2%) had a recurrence [Table 2]. Recurrence occurred in a higher proportion of AR-negative group ($P = 0.458$). Recurrence-free survival was found to be higher on plotting the Kaplan–Meier survival curve for AR-positive group (log-rank test $P = 0.342$). A higher proportion of ERβ-positive (41.7%) group showed recurrence ($P = 0.533$) [Table 3]. The median (IQR) of time to recurrence (months) in the AR-positive group was 18 (6–44) months while in the AR-negative group, it was 13 (8–33.25) months. The median (IQR) of time to recurrence (months) in the ERβ-positive group was 12.5 (7.75–30.75) months while in the ERβ-negative group, it was 18 (9–34) months.

Among nine patients with AR-positive tumor, one patient (11.1%) had tumor progression. Among 36 patients with ERβ-positive tumor, two (5.6%) had a progression. PFS was found to be significantly lower on plotting Kaplan–Meier survival curve for ERβ-negative group (log-rank test $P = 0.035$) [Supplementary Figure 3]. The median (IQR) of time to progression (months) in the AR-positive group was 18 (12–45) months while in the ERβ-positive group, it was 16 (8.75–42.5) months.

Table 2: The association between the clinical and pathological characteristics of patients according to androgen receptor expression status

Characteristics	AR (positive)	AR (negative)	*P*
Sex			
Male	8	33	0.420
Female	1	15	
Pathologic T stage			
Ta	4	31	0.286
T1	5	17	
Pathologic grade			
Low grade	6	35	0.700
High grade	3	12	
Recurrence			
Yes	2	20	0.458
No	7	28	
Progression			
Yes	1	5	1.000
No	8	43	

AR = Androgen receptor

Table 3: The association between the clinical and pathological characteristics of patients according to estrogen receptor beta expression status

Characteristics	ERβ (positive)	ERβ (negative)	*P*
Sex			
Male	24	17	0.247
Female	12	4	
Pathologic T stage			
Ta	21	14	0.533
T1	15	7	
Pathologic grade			
Low grade	26	15	0.949
High grade	10	6	
Recurrence			
Yes	15	7	0.533
No	21	14	
Progression			
Yes	2	4	0.179
No	34	17	

ER = Estrogen receptor

DISCUSSION

In the current study, 22 patients (38.6%) received intravesical therapy which is reflective of the T1 burden of the disease in the cohort. The proportion of patients who did not receive intravesical therapy showed a higher recurrence rate than patients who received intravesical therapy (45.7% vs. 27.3%, $P = 0.164$), although this difference did not reach a statistical significance.

In the Indian population, men are described to have nine times more likelihood of developing bladder cancer than women. Significant gender difference in smoking distribution was found in the current study, among 21 patients who had a history of smoking 18 patients (85.7%) were male while only three patients (14.3%) were female. This difference in the gender distribution of bladder cancer and smoking has been reported in various studies. In a study by Freedman *et al.*,[10] smoking was attributed to 50%–65% of male bladder cancer cases and 20%–30% of female cases.

In the current study, a higher proportion of females presented with a higher stage (T1, 43.8% vs. 36.6%) and higher grade (HG 37.5% vs. LG 24.4%) compared to males. However, this difference between the two groups was not statistically significant ($P = 0.618$ and $P = 0.343$, respectively). Furthermore, a higher proportion of females had progression (18.8% vs. 7.3%, $P = 0.355$) with lower mean time to progression (25.67 [23.03] months vs. 47.33 [10.26] months, $P = 0.241$). It has been found in various clinical studies that women present with a higher-stage tumor and tend to have a more aggressive tumor.[1,2] Tumor biology, environmental exposure, and delay in diagnosis among females have been attributed to this gender difference.[11,12]

In addition to the above-mentioned reasons for gender disparity, steroid hormones, especially androgen and estrogens and AR/ERs, have found to have a critical role in bladder tumorigenesis and progression.[4,5] A number of immunohistochemical studies have shown varied

expressions of steroid receptors depending on tumor stage and grade. A comparison of the proportion positivity of AR in total sample size, gender, and tumor stage with previous studies is shown in Supplementary Table 2.

Most of the studies have shown downregulation of AR with increasing stage of tumor.[13,14] However, a study by Mir *et al.*[15] has shown that there is no decrease in the expression of AR with increasing pathological stage or grade. In the current study, no statistically significant difference in the distribution of AR was found in different stages and grades of tumor (P = 0.286 and P = 0.700) [Table 2]. In our study also, AR-negative tumors showed much higher recurrence (20/48 = 42%) as compared to AR-positive (2/9 = 22%) tumors, although this did not reach a statistical significance (P = 0.458) [Table 2 and Supplementary Table 3]. This could be attributed to the fact stated by existing literature that downregulation of AR may be associated with increasing stage and probably increasing recurrence rates.[16,17] However, this trend was not reflected in the progression rate, time to recurrence, and time to progression.

In the current study, ERα stained positive for only one patient who was male in a T1 HG tumor. Most of the studies have shown similar results with ERα positive staining for a small subset (1%–5%) of bladder tumor specimens.[16,18] However, in another large study by Miyamoto *et al.*,[17] higher positivity for ERα was found (27%). As ERα positivity was low in the current study, further analytical analysis for ERα was omitted.

A comparison of the proportion positivity of ERβ in total sample size, gender, and tumor stage with previous studies is shown in Supplementary Table 4. Most of the studies, as shown in Supplementary Table 4, have shown upregulation of ERβ with increasing stage and grade of tumor.[12,16,17] However, a study by Kontos *et al.*[19] has shown downregulation of ERβ with increasing pathological stage or grade. In the current study, no significant difference in the distribution of ERβ was found in different stages and grades of NMIBC (P = 0.533 and P = 0.949) [Table 3 and Supplementary Table 4].

In our study, ERβ-positive tumors showed much higher recurrence (42%) as compared to ERβ-negative (33%) tumors although this did not reach a statistical significance [Supplementary Table 5]. The difference between ERβ-positive and negative groups was not significant (16 months vs. 20 months, Wilcoxon-Mann–Whitney U Test, P = 0.803). However, PFS was found to be significantly lower on plotting the Kaplan–Meier survival curve for ERβ-negative group (log-rank test P = 0.035). Most of the studies have found a higher likelihood of recurrence and progression in ERβ-positive group.[8,20] Since the sample size of the current study was small, and more events of progression were found in ERβ-negative group (4 vs. 2), data were skewed toward ERβ-negative group on the Kaplan–Meier survival analysis, which might be the reason for the observed lower PFS in the current study. The limitation of our study included a small sample size due to the limitation of funding for kits. Further, intravesical therapy and follow-up duration were not uniform in this study. However, to the best of our knowledge of English literature, this is the first study on steroid receptors in a uniform cohort of NMIBC patients with mid-term follow-up.

CONCLUSION

Steroid receptors (AR and ERβ) positivity is seen in patients with NMIBC. Although it did not reach a statistical significance, a higher proportion of AR-negative and ERβ-positive tumors recurred as compared to AR-positive and ERβ-negative patients. However, PFS was significantly lower in ERβ-negative group. Further exploratory studies on larger sample sizes are required to validate these findings in NMIBC patients.

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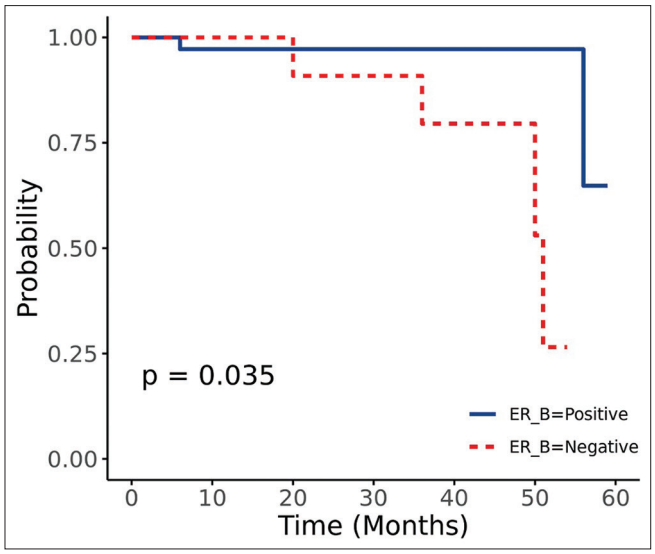
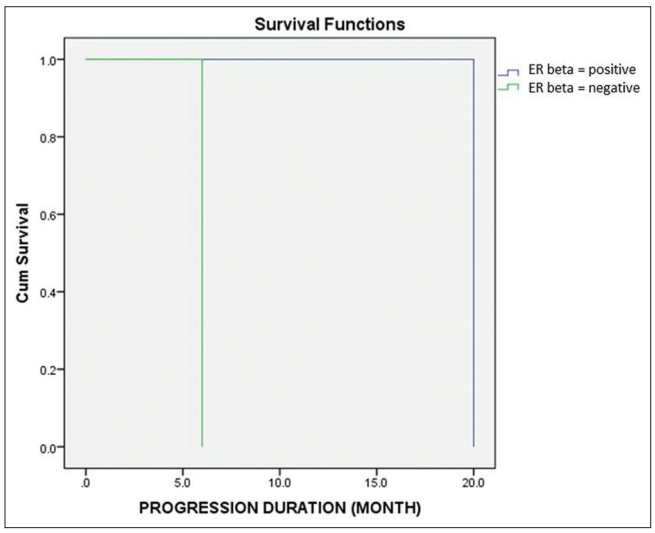
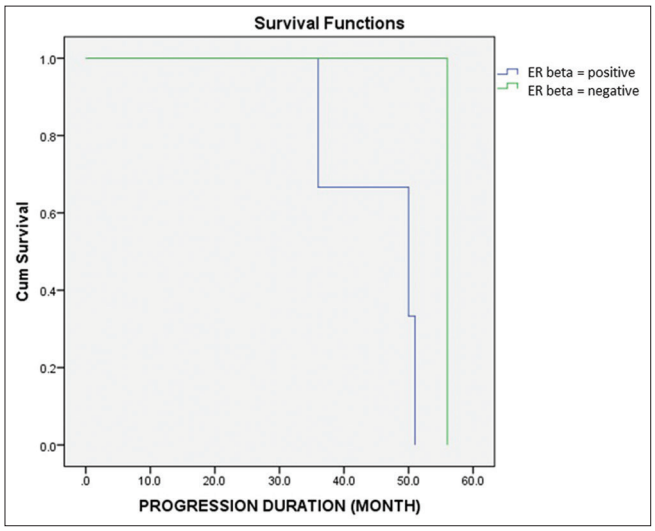


Supplementary Figure 1: Kaplan–Meier curve showing lower progression-free survival for ERβ-negative group



Supplementary Figure 2: Kaplan–Meier curve showing progression-free survival in patients who received intravesical therapy



Supplementary Figure 3: Kaplan–Meier curve showing progression-free survival in patients who did not receive intravesical therapy

Supplementary Table 1: Distribution of receptor positive patients according to their stage and grade

Receptor	Ta LG (*n*=35), *n* (%)	T1 LG (*n*=6), *n* (%)	T1 HG (*n*=16), *n* (%)
AR (positive)	4 (11.4)	2 (33.3)	3 (18.8)
ERα (positive)	-	-	1 (6.25)
ERβ (positive)	21 (60)	5 (83.3)	10 (62.5)

AR=Androgen receptor, ER=Estrogen receptor, LG=Low-grade, HG=High-grade

Supplementary Table 2: Comparison of androgen receptor expression

Study	Patients (*n*)	Gender (*n*)		AR, *n* (%)	Gender distribution (AR positive)		*P*	NMIBC (AR positive)		*P*
		Male (%)	Female (%)		Male (%)	Female (%)		Ta	T1	
Boorijan[14] (2004)*	49	36	13	26 (53.1)	61	31	0.104	88.9	76.9	0.005
Miyamoto[17] (2012)*	188	148	40	109 (58)	42	43	1.000	53	17	0.018
Mir[15] (2011)*	472	386	86	61 (12.9)	14	8	0.159	11.8	5.2	0.086
Nam[16] (2014)	169	143	26	63 (37.2)	38	31	0.303	43.4	29.9	0.048
Jing[13] (2014)*	58	44	14	31 (53)	57	43	0.540	49		0.225
Current study	57	41	16	9 (15.8)	19.5	6.2	0.420	11.4	22.7	0.286

*Study including patients with both NMIBC and MIBC. MIBC=Muscle-invasive bladder cancer, NMIBC=Non-MIBC, AR=Androgen receptor

Supplementary Table 3: Comparison of androgen receptor expression with recurrence and progression

Study	Patients (*n*)	Mean follow up duration (range)	AR	Recurrence, *n* (%)	*P*	Progression, *n* (%)	*P*
Nam[16] (2014)	169	53.7 (25–82) months	AR (positive)	19 (30.2)	0.002	8 (12.7)	0.205
			AR (negative)	57 (53.7)		20 (18.9)	
Current study	57	18 (9–44) months	AR (positive)	2 (22.2)	0.458	1 (11.1)	1.000
			AR (negative)	20 (41.7)		5 (10.4)	

AR=Androgen receptor

Supplementary Table 4: Comparison of estrogen receptor β expression

Study	Patients (*n*)	Gender (*n*)		ERβ, *n* (%)	Gender (ERβ positive)		*P*	NMIBC (ERβ positive)		*P*
		Male (%)	Female (%)		Male (%)	Female (%)		Ta (%)	T1 (%)	
Shen[21] (2006)*	224	NA		141 (63)		NA		53	58	<0.001
Miyamoto[17] (2012)*	188	148	40	93 (49)	53	38	0.109	32	67	<0.001
Nam[16] (2014)	169	143	26	52 (30.7)	30.7	30.7	0.583	21.7	41.5	<0.004
Tan[22] (2015)*	318	259	59	318 (100)			100			-
Kontos[19] (2010)*	140	94	46	76.6		NA			84	<0.001
Current study	57	41	16	36 (63.2)	58.5	75	0.247	60	68.2	0.533

*Study including patients with both NMIBC and MIBC. MIBC=Muscle-invasive bladder cancer, NMIBC=Non-MIBC, AR=Androgen receptor, ER=Estrogen receptor, NA=Not available

Supplementary Table 5: Comparison of estrogen receptor β expression with recurrence and progression

Study	Patients (*n*)	Mean follow-up duration (range)	ERβ	Recurrence, *n* (%)	*P*	Progression, *n* (%)	*P*
Nam[16] (2014)	169	53.7 (25–82) months	ERβ (positive)	31 (59.6)	0.009	14 (26.9)	0.016
			ERβ (negative)	45 (38.4)		14 (11.9)	
Current study	57	18 (9–44) months	ERβ (positive)	15 (41.7)	0.533	2 (5.6)	0.179
			ERβ (negative)	7 (33.3)		4 (19)	

ER=Estrogen receptor