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Laboratory-Bladder cancer

Sex steroid hormone receptors in bladder cancer: Usefulness in differential diagnosis and implications in histogenesis of bladder cancer

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Abstract

Objective: In rare cases, differential diagnosis between bladder cancer (BC) and gynecological tract cancer (GTC) is difficult because of anatomical proximity and morphological similarity. We analyzed expression status of sex steroid hormone receptors in BC in this study. First, we investigated their usefulness as a histological marker for differential diagnosis. Second, we considered their roles in BC histogenesis.

Methods: Estrogen receptor α (ERα) and progesterone receptor (PgR) expression was investigated by immunohistochemistry in 125 BCs obtained by transurethral resection or biopsy, then in nonneoplastic background mucosa (trigone, fundus, and dome) of 33 total cystectomy samples. They were evaluated as positive when $\geq$ 1% of 500 subject cells were immunoreactive with moderate or strong intensities.

Results: ERα and PgR were positive in 38.4% and 3.2% of BCs, respectively, suggesting that ERα status alone could not definitely differentiate between BC and GTC. ERα expression was not significantly associated with age and sex of BC patients and histopathology of BCs. Although not significant, ERα expression was more frequent in higher grade (G1/G2 vs. G3/G4; $P = 0.143$) and marginally associated with advanced stage of BCs (pTis/pTa/pT1 vs. pT2/pT3, $P = 0.056$). ERα expression was significantly more frequent in background mucosa with ERα-positive BC (In the epithelium and stroma; both $P < 0.001$). ERα expression was continuously observed from normal to malignant epithelium in some cases. Although not significant, Brunn's nest or cystitis glandularis was more frequent in background mucosa with ERα-positive BC ($P = 0.218$). Analyses of nonneoplastic mucosa in cystectomy revealed that ERα was more frequently positive in urothelium of trigone, a predilection site for cystitis glandularis, than those of fundus and dome, with a significant difference between trigone and dome ($P = 0.034$). These data suggest that chronic inflammation may up-regulate ERα in the background epithelium, especially in trigone, and ERα expression in BC might be the reflection of bladder epithelium from which BC arose.

Conclusions: Usefulness of ERα was limited in differential diagnosis between BC and GTC. ERα up-regulation might not play a critical role in the development of BC because it was already noted in the background bladder mucosa.

Keywords: Bladder cancer; Uterine cancer; Estrogen receptor; Differential diagnosis; Immunohistochemistry; Histogenesis

1. Introduction

Diagnosis of primary bladder cancer (BC) is easy in most cases. However, owing to its anatomical location, it may sometimes invade the gynecological tract [1]. When this occurs, it is difficult to accurately determine the primary origin of cancer in the pelvic cavity [1]. Furthermore, there is a significant morphological overlap between primary gynecological lesions and invasive urothelial carcinoma (UC) [1–3].

Immunohistochemical cytokeratin (CK) 7 and CK20 profiles have been introduced to investigate the primary site of epithelial neoplasms [4], but differential diagnosis solely based on CK7/CK20 profiles may sometimes result in misdiagnosis. Additional markers, such as p16/INK4α/CDKN2A, and in situ hybridization (ISH) for human papilloma virus (HPV) have been introduced for differentiation diagnosis

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between UC and gynecological tract cancer (GTC) [1]. p16 up-regulation is associated with infection of high-risk HPV, and endocervical carcinoma is mostly p16-positive [5]. However, p16 was diffusely positive in 3 of 6 cases of UC that involved the gynecological tract, suggesting p16's limited utility. HPV ISH was negative in all 6 cases, but it is available only in limited facilities.

Uterus is a major target of female sex steroid hormones but urinary bladder is apparently not a common target. In this study, we noted estrogen receptor α (ERα) and progesterone receptor (PgR) in BC in a Japanese population as a possible histological marker for differential diagnosis. During this study, we noticed that ERα is frequently positive in the background bladder mucosa in ERα-positive BC cases. Our secondary goal with this study was to consider the implication of ERα expression during bladder carcinogenesis. We hypothesized that ERα up-regulation may not play a critical role if it was frequently expressed in nonneoplastic bladder mucosa. To test the hypothesis, we additionally performed comprehensive expression analyses of ERα in the normal mucosa of cystectomy samples.

2. Materials and methods

2.1. Study subjects

We investigated ERα and PgR expression by immunohistochemistry (IHC) in 125 BCs, consisting of 93 transurethral resections (TUR) or biopsy specimens from 83 patients from 2014 to 2015 and 32 cystectomy samples from 32 patients between 2002 and 2015 in Dokkyo Medical University Hospital. Eight patients underwent TUR or biopsy twice and 1 patient did so 3 times. Two patients underwent cystectomy after biopsy or TUR.

Inclusion criteria were the presence of glass slides, which included carcinoma with an area of >1.0 cm^2 and absence of concomitant renal pelvis cancer and urachal cancer. Histopathological diagnosis was based on the World Health Organization (WHO) classification of tumors of the urinary system and male genital organs, fourth edition [6] except for histopathological grading which was performed according to the TNM classification of malignant tumors, seventh edition [7]. The pathological extent of tumor (pT) was determined in the observable range of TUR or biopsy specimens. All sections were reviewed for confirmation of original diagnoses by an expert pathologist (Y.I.). Clinicopathologic characteristics of the patients were obtained via an electronic medical chart system.

Next, we performed comprehensive analyses of background bladder mucosa, i.e., dome, fundus, and trigone, using 33 total cystectomy samples from 25 male and 8 female patients between 2002 and 2015.

Written informed consent to use resected samples for medical research was obtained from patients by their surgeons. This study protocol was approved by the institutional ethics review board (No. 28042).

2.2. IHC

Tumor specimens were fixed in 10% neutral-buffered formalin for 48 hours, embedded in paraffin, then cut into 4-μm sections. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) using microwave irradiation (400 W) at 95°C for 40 minutes. After quenching endogenous peroxidase activity, sections were incubated with primary antibody detecting ERα (1:40, clone 6F11; Novocastra, Newcastle upon Tyne, UK) or PgR (1:100, clone 16; Novocastra) for 60 minutes at room temperature. All these stains were manually scored by 1 pathologist (Y.I.) who was blinded to patient identity at the time of scoring. Each receptor was evaluated as positive when $\geq$1% of 500 subject cells were immunoreactive with moderate or strong intensities, which corresponded to 1+ or more in the German immunoreactive score [8].

2.3. Statistics

Specific parameters between 2 datasets were compared using the Fisher's exact test. Age was compared using the Mann–Whitney U test. In patients with many times of biopsies and resections, age at the time of ERα-positive or earlier ones with the same ERα status were analyzed. Hormone receptor levels in the respective segments of background bladder mucosa were compared by the Wilcoxon signed-rank test. $P < 0.05$ was considered significant. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM, Armonk, NY).

3. Results

3.1. ERα and PgR expression in BC

The 125 BCs consisted of 100 noninvasive/invasive UC, 20 invasive UC, special types (UCS), with components such as glandular or squamous differentiation, plasmacytoid type, sarcomatoid type, giant cell type, poorly differentiated type, and five miscellaneous types (MIS) consisting of pure adenocarcinoma (AD), pure squamous cell carcinoma (SCC), and neuroendocrine carcinoma. ERα and PgR were positive in 48 (38.4%) and 4 (3.2%) cancers, respectively (Fig. 1A and B) (Table 1). ERα was positive in 9 of 20 (45.0%) UC and 3 of 6 (50.0%) UCS/MIS in female patients and 28 of 80 (35.0%) UC and 8 of 19 (42.1%) UCS/MIS in male patients. There were no significant differences in age and sex between ERα-positive and -negative patients (Table 2). ERα expression was more frequent in pT2/pT3 BCs than in pTis/pTa/pT1 ones with marginal significance (45.8% *vs.* 28.6%, $P = 0.056$) (Table 3). There were no significant differences in histopathology and grade according to the ERα expression status, although high-grade (G3/G4) was more frequent than low-grade (G1/G2) in ERα-positive BC (Table 3). Because it was difficult to precisely determine the original site of BC in fragmented TUR samples and cystectomy samples post TUR, the

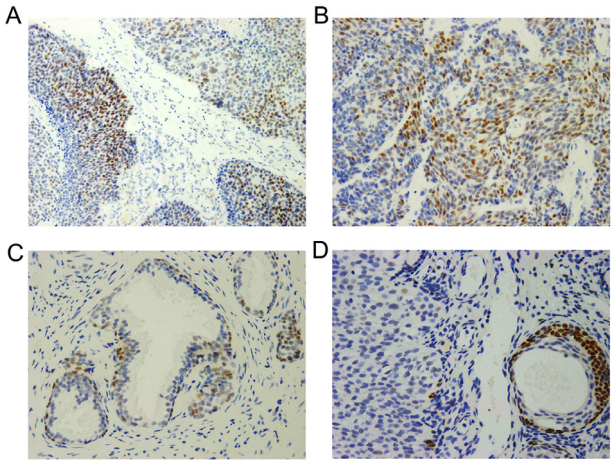

Fig. 1. (A) ERα expression in invasive UC (Case 70, ×4). (B) PgR expression in invasive UC (Case 53, ×20). (C) ERα expression in the background mucosa presenting with cystitis glandularis (Case 11, ×20). (D) Continuous ERα expression from the nonneoplastic mucosa to noninvasive UC (Case 21, ×20).

Table 1
Expression of sex steroid hormone receptors in BC

	Grade	BC	
		ERα (+)	PgR (+)
UC (n = 100)	G1 (n = 4)	0	0
	G2 (n = 55)	18	1
	G3 (n = 41)	19	0
UCS (n = 20)	G2 (n = 3)	1	0
	G3 (n = 12)	6	0
	G4 (n = 5)	1	0
MIS (n = 5)	G2 (n = 1)	1	1
	G3 (n = 4)	2	2
Total (n = 125)		48	4

BC = bladder cancer; ERα = estrogen receptor α; MIS = miscellaneous types; PgR = progesterone receptor; UC = urothelial carcinoma; UCS = urothelial carcinoma, special types. Grade was based on TNM seventh edition.

Table 2
ERα expression and clinicopathologic characteristics of patients with BC

		BC		
		ERα (−)	ERα (+)	*P* value
Age	Range	37-93	47-91	0.916
	Median	70	71	
Sex	Male	56	33	0.347
	Female	12	12	

BC = bladder cancer; ERα = estrogen receptor α. In cases of patients with many times of biopsies, data at the time of ERα (+) or earlier ones with the same ERα status were analyzed.

association between ERα expression and tumor location could not be analyzed.

During investigation, we noticed that ERα expression might be frequent in the background mucosa with ERα-positive BC and we therefore investigated that association. ERα was positive in nonneoplastic epithelium in 10.9% of ERα-negative BC cases and 73.0% of ERα-positive BC cases (Fig. 1C), and in stromal cells in 45.5% of ERα-negative BC cases and 77.1% of ERα-positive BC cases, respectively. Some of the ERα-positive epithelium presented with cystitis glandularis or squamous metaplasia. Accurate discrimination between squamous epithelium and urothelial epithelium was often difficult because of the transitional feature between them. Thus, ERα-positive BC was accompanied by the ERα-positive background mucosa both in the urothelium and stroma with statistical significance (both $P < 0.001$). ERα expression was continuously observed from the nonneoplastic to neoplastic epithelium in some cases (Fig. 1D). In addition, ERα expression in nonneoplastic urothelium and inverted urothelial papilloma that coexisted with UC was also noted in one case (Case 82; Fig. 2). Brunn's nest and/or cystitis glandularis in the background mucosa were noted in 13.0% of ERα-negative BCs and 22.9% of ERα-positive BCs, though this increased frequency was not significant. These data are summarized in Table 3.

Table 3
ERα expression and clinicopathologic characteristics of BC

	BC		
	ERα (−)	ERα (+)	*P* value
Total	77	48	N.A.
pT			
pTis, pTa	21	11	0.056 (pTis, pTa, pT1 vs. pT2, pT3)
pT1	34	15	
pT2	15	21	
pT3	7	1	
Grade			
G1	4	0	0.143 (G1, G2 vs. G3, G4)
G2	39	20	
G3	30	27	
G4	4	1	
Histopathology			
UC	63	37	0.646
others	14	11	
Background epithelium			
ERα expression			
ERα (−)	49	10	0.000
ERα (+)	6	27	
Unknown	22	11	
Brunn's nest or cystitis glandularis			
Absent	67	37	0.218
Present	10	11	
Background stroma			
ERα (−)	42	11	0.0007
ERα (+)	35	37	

BC = bladder cancer; ERα = estrogen receptor α; MIS = miscellaneous types; N.A. = not applicable; PgR = progesterone receptor; UC = urothelial carcinoma; UCS = urothelial carcinoma, special types. pT and grade was determined based on WHO fourth edition and TNM seventh edition, respectively.

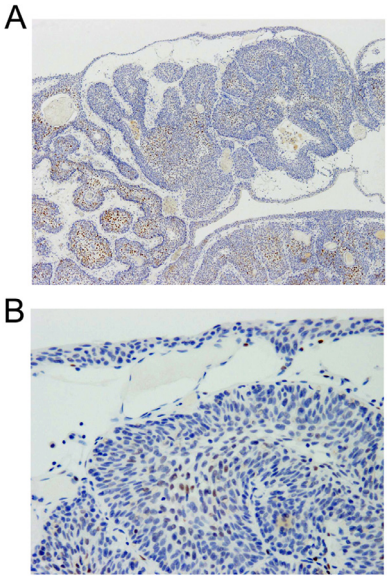

Fig. 2. ERα expression in the non-neoplastic urothelium and inverted urothelial papilloma (Case 82). (A) ×4. (B) ×20.

PgR was positive only in four cases (Table 4), all of which were men. Three of the 4 cancers were not UC, 2 were SCC and 1 was small cell neuroendocrine carcinoma. PgR expression in the adjoining nonneoplastic mucosa was observed only in 1 case.

3.2. ERα expression in the background mucosa of cystectomy samples

We then investigated ERα expression in nonneoplastic background mucosa using 33 whole cystectomy samples, 25 of which were from men. ERα and PgR were positive in the background epithelium in 57.6% and 21.2%, respectively, and in the background stroma in 84.8% and 100%, respectively. ERα expression in epithelium was more frequent in trigone than fundus and dome (46.9%, 31.0%, and 22.2%, respectively) with a significant difference between trigone and dome ($P = 0.034$) (Table 5).

Table 4
Cases of BC with PgR expression

Case No.	50	53	82	115
Sex	Male	Male	Male	Male
Age	71	85	64	54
Histopathology	SCC	SCC	UC	Small-cell neuroendocrine carcinoma
pT	pTa	pT2	pTa	pT2
Grade	G2	G3	G2	G3
ERα	(+)	(−)	(+)	(−)
Background epithelium				
ERα	(+)	(−)	(+)	(−)
PgR	(+)	(−)	(−)	(−)
Background stroma				
ERα	(+)	(−)	(+)	(+)
PgR	(+)	(−)	(+)	(+)

BC = bladder cancer; ERα = estrogen receptor α; PgR = progesterone receptor; UC = urothelial carcinoma; SCC = squamous cell carcinoma. pT was and grade was based on WHO 4th edition and TNM 7th edition, respectively.

4. Discussion

BC sometimes involves gynecological organs because of its anatomical location, which may pose diagnostic difficulty between BC and GTC clinically; significant morphologic overlap exists between UC and GTC [1−3]. The common histological architecture seen in UC, such as papillary, trabecular, and solid growth patterns are also observed in endocervical carcinoma [1,3]. High-grade UC, UC with glandular differentiation, and bladder AD may mimic endometrial carcinoma [2]. In very rare cases, endometrioid endometrial carcinoma may arise from endometriosis of the urinary bladder [9]. Usefulness of the CK7/CK20 pattern is often limited for differentiation diagnosis, so we noted ERα and PgR as possible histologic markers for differential diagnosis between BC and GTC.

ERα expression status in BC has not yet been elucidated sufficiently, especially in an Asian population. Because the urinary bladder seemed not to be a major target of sex steroid hormones, we initially expected a low-expression rate. In Western countries, several groups have analyzed ERα expression in BC by IHC [8,10−16]. ERα-positive rate of UC was 10∼30% according to the German score (Table 6). In this study, ERα was positive in 38.4% of BC in a Japanese population by the German score. Thus, the ERα-positive rate was higher than previous reports. For women, nearly 50% of BC was positive for ERα expression. Regarding GTC, approximately 80% of endometrioid endometrial cancers are ERα positive (≥1% tumor cell positivity), irrespective of tumor grade [17]. In contrast, expression of ERα decreases or vanishes in endocervical cancer. ERα is strongly expressed in the nuclei of cervical squamous epithelial cells mainly in parabasal layers, but ERα expression was not observed in any invasive endocervical SCC [18−20]. In other studies, ERα expression was found only in 9.5% [21] and 18.2% [22] of invasive endocervical SCC. The cutoff for ERα positivity was not documented by Nonogaki et al. [21] but was ≥1% tumor cell positivity by Kanai et al. [22]. ERα was expressed at least focally in 12% of endocervical AD, usual-type [23]. Taken together, ERα expression might favor BC over endocervical cancer and the absence of ERα expression might favor BC over uterine corpus cancer. However, ERα alone was not a definite determining factor of differentiation diagnosis because all of the BC, endometrial cancer, and endocervical cancer could express ERα with various degrees of frequency. Combining ERα expression with other markers would be mandatory for definite diagnosis.

The previous studies that assessed the relationship between ERα expression and histopathological characteristics of BC have led to conflicting results (Table 6). In this

Table 5
ERα and PgR expression in the background bladder mucosa of total cystectomy samples

	ERα (−)	ERα (+)	Total	*P* value (vs. trigone)	PgR (−)	PgR (+)	Total	*P* value (vs. trigone)
Epithelium								
trigone	17	15	32		27	5	32	
fundus	20	9	29	0.317	28	1	29	0.102
dome	21	6	27	0.034	26	1	27	0.102
whole	14	19	33	N.A.	26	7	33	N.A.
Stroma								
trigone	12	20	32		0	32	32	
fundus	12	17	29	0.763	0	29	29	1.000
dome	11	16	27	0.480	0	27	27	1.000
whole	5	28	33	N.A.	0	33	33	N.A.

ERα = estrogen receptor α; PgR = progesterone receptor; N.A. = not applicable.

study, ERα was positive more frequently in higher grade and invasive UC. Consistent with our results, the ERα-positive rate was significantly more frequent in higher grade and/or invasive UC [10–12], but ERα expression was not associated with prognosis [11]. In contrast, association of ERα expression with organ confined tumor stage of UC [14] and association between loss of ERα expression and higher grade/more invasive UC [8] have been reported, but ERα expression was not associated with prognosis. We speculate that these conflicting results might be due to differences in epidemiological factors, sample size, study methods, and evaluation criteria for ERα/PgR positivity. In particular, IHC procedures may greatly affect the results. Unlike a biochemical assay, IHC has an advantage of detecting sex steroid hormone receptor expression in respective mucosal components such as epithelium and stromal tissue. However, there are many differences in experimental procedures, such as antigen retrieval technique, antibodies used, and evaluation criteria among previous studies and the present study. Interobserver variability in evaluation of signal intensities may also exist. Any of these factors would cause inconsistent results of ERα positivity between studies.

Reportedly, ERα was positive in 50% of benign urothelium and 67% of benign stroma, respectively [8]. In this study, ERα expression rate in the background epithelium and stroma was significantly higher in ERα-positive BC cases than in ERα-negative BC cases. Although not statistically significant, the Brunn's nest or cystitis glandularis rates were higher in ERα-positive BC cases than in ERα-negative BC cases. Brunn's nest, cystitis glandularis, and squamous metaplasia may result from chronic inflammation [24,25] and most often found in the trigone area [26]. In addition, ERα was frequently expressed in the epithelium of trigone but not at all in dome of the female bladder [27]. ERα was consistently expressed in urothelial epithelium undergoing squamous metaplasia [27]. In this study, analyses of total cystectomy samples, 75.8% of which were from men, also revealed that ERα and PgR was expressed in 59.3% and 21.9% of the background bladder epithelium, respectively, and that ERα expression was more frequent in trigone as compared with fundus and dome. Taken together, we speculate that chronic inflammation may affect ERα expression in the background epithelium, and ERα expression in BCs might be a reflection of the bladder epithelium from which they arose. Continuous ERα expression from the nonneoplastic to neoplastic epithelium and ERα expression in the background urothelium and inverted urothelial papilloma that coexisted with UC observed in the present study may support this hypothesis. In the previous studies [10–12] and the present study, ERα expression tended to be associated with more advanced pT stage and higher grade, which might be associated with stronger inflammation owing to the immune reaction and/or repeated TUR against cancer. Recently, an interesting study was published by Bernardo, et al. [16]. In schistosomiasis-associated UCs, 22% of tumor cells were ERα positive, and the presence of parasite eggs was associated with higher ERα expression in cancer cells and the adjacent urothelium. This report may also support the association between ERα expression and inflammation in the primary urothelium.

Preclinical studies reporting the roles of sex hormone receptor signals on BC have been inconsistent. Bernardo, et al. reported that proliferation of BC cells with ERα expression but not with ERβ expression was stimulated by estradiol [16]. Teng, et al. also reported that ERα may play a role in the proliferation of BC cells by inducing phosphorylation of extracellular signal-regulated kinases and up-regulation of cyclins D1 and E during the cell cycle [28]. In contrast, Hsu, et al. reported that ERα inhibited BC development and loss of ERα resulted in carcinogen-induced BC by modulating AKT activity in an ERα-knockout mouse model [29]. They also showed that ERα-knockdown in BC cell lines had a growth advantage over control cells [29]. The reason for this discrepancy is presently unknown. We speculate that differences in experimental conditions, cell lines and ERα expression levels, and study subjects might have affected the results.

Table 6
Previous analyses of sex steroid hormone receptor expression in BC by immunohistochemistry

	N	Histology	Ethnicity	ERα positive rate	Effect of positive ERα			ERβ positive rate	PgR positive rate	Standard for positivity
					Grade	Stage	Prognosis			
Kaufmann et al. [10]	185	UC	Germany	18%	Higher	Higher	N.A.	N.A.	N.A.	German score
Basakci et al. [11]	121	UC	Turkey	12.4%	Higher	N.A.	None	N.A.	N.A.	German score
Croft et al. [12]	92	UC	USA	11%	N.A.	N.A.	N.A.	N.A.	N.A.	>10%
Croft et al. [12]	92	UC	USA	22%	Higher	Higher	N.A.	N.A.	N.A.	>0%
Shen et al. [13]	224	UC	USA	0.9%	N.A.	N.A.	N.A.	63%	N.A.	>10%
Bolentz et al. [14]	198	UC	USA	4.5%	N.A.	Lower	None	N.A.	0%	Not described
Miyamoto et al. [8]	188	UC	USA	27%	Lower	Lower	None	49%	N.A.	German score
Mashhadi et al. [15]	252	UC	Iran	4.2%	N.A.	N.A.	N.A.	N.A.	2.5%	>10%
Bernard et al. [16]	107	UC	Portugal	18.7%	Higher	N.A.	Poor	87.9%	N.A.	Not described
Present study	125	100 UC, 20 UCS, 5 MIS	Japan	38.4%	None	Marginally higher	N.A.	N.A.	3.2%	German score

BC = bladder cancer; ERα = estrogen receptor α; MIS = miscellaneous types; N.A. = not applicable; PgR = progesterone receptor; UC = urothelial carcinoma; UCS = urothelial carcinoma, special types.

Because of the small number of PgR-positive BCs, we could not draw any definite conclusion regarding its clinicopathologic significance. It is noteworthy, however, that most PgR-positive cases were not typical UC. PgR expression might be associated with the histological transformation to UCS.

5. Conclusion

ERα and PgR expression was observed in 38.4% and 3.2% of BCs, respectively. ERα expression was found in more advanced BCs. The presence of ERα expression might be partly useful in differential diagnosis from GTC, but ERα alone could not be a definite determining factor of differentiation diagnosis. In addition, our results suggested that ERα up-regulation might not play a critical role in the development of BC because it was noted in the background bladder mucosa from which BC arose.

References

[1] Reyes MC, Park KJ, Lin O, Ioffe O, Isacson C, Soslow RA, et al. Urothelial carcinoma involving the gynecologic tract: A morphologic and immunohistochemical study of 6 cases. Am J Surg Pathol 2012;36:1058–65. https://doi.org/10.1097/PAS.0b013e318251eade.

[2] Mirsadraei L, Hodkoff A, Jones K, Shabaik A, Kader AK, Saenz CC, et al. Serous carcinoma mimicking primary urothelial carcinoma on clinical evaluation and pathology: A potential diagnostic pitfall. Arch Pathol Lab Med 2018;142:168–77. https://doi.org/10.5858/arpa.2017-0004-OA.

[3] Sams SB, Rosser JA. Metastatic urothelial carcinoma to the uterine cervix: A case report of a rare entity and review of the diagnostic differential. Int J Gynecol Pathol 2017;36:493–8. https://doi.org/10.1097/PGP.0000000000000343.

[4] Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: A survey of 435 cases. Mod Pathol 2000;13:962–72. https://doi.org/10.1038/modpathol.3880175.

[5] O'Neill CJ, McCluggage WG. p16 expression in the female genital tract and its value in diagnosis. Adv Anat Pathol 2006;13:8–15. https://doi.org/10.1097/01.pap.0000201828.92719.f3.

[6] TNM classification of the urinary bladder. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE, eds. WHO Classification of Tumors of the Urinary System and Male Genital Organs, 4th edition, Lyon: IARC Press; 2016. p. 79.

[7] Gospodarowicz M. Urinary bladder: Rules for classification. In: Sobin L, Gospodarowicz M, Wittekind C, eds. TNM Classification of Malignant Tumors, 7th edition, West Sussex: Wiley-Blackwell; 2009. p. 264.

[8] Miyamoto H, Yao JL, Chaux A, Zheng Y, Hsu I, Izumi K, et al. Expression of androgen and estrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. BJU Int 2012;109:1716–26. https://doi.org/10.1111/j.1464-410X.2011.10706.x.

[9] Sarfraz A, Aitken L, Wilson J, Byrne D. Endometrioid endometrial carcinoma of the urinary bladder – A diagnostic challenge. J Med Diagn Meth 2014;3:2. https://doi.org/10.4172/2168-9784.1000155.

[10] Kaufmann O, Baume H, Dietel M. J Pathol. Detection of oestrogen receptors in noninvasive and invasive transitional cell carcinomas of the urinary bladder using both conventional immunohistochemistry and the tyramide staining amplification (TSA) technique. J Pathol 1998;186:165–8. https://doi.org/10.1002/(SICI)1096-9896(1998100)186:2<165::AID-PATH155>3.0.CO;2-Y.

[11] Basakci A, Kirkali Z, Tuzel E, Yorukoglu K, Mungan MU, Sade M. Prognostic significance of estrogen receptor expression in superficial

transitional cell carcinoma of the urinary bladder. Eur Urol 2002;41:342–5.

[12] Croft PR, Lathrop SL, Feddersen RM, Joste NE. Estrogen receptor expression in papillary urothelial carcinoma of the bladder and ovarian transitional cell carcinoma. Arch Pathol Lab Med 2005;129:194–9. https://doi.org/10.1043/1543-2165(2005)129<194:EREIPU>2.0.CO;2.

[13] Shen SS, Smith CL, Hsieh JT, Yu J, Kim IY, Jian W, et al. Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue. Cancer 2006;106:2610–6:http://doi.org/0.1002/cncr.21945.

[14] Bolenz C, Lotan Y, Ashfaq R, Shariat SF. Estrogen and progesterone hormonal receptor expression in urothelial carcinoma of the bladder. Eur Urol 2009;56:1093–5. https://doi.org/10.1016/j.eururo.2009.06.032.

[15] Mashhadi R, Pourmand G, Kosari F, Mehrsai A, Salem S, Pourmand MR, et al. Role of steroid hormone receptors in formation and progression of bladder carcinoma: A case-control study. Urol J 2014;11:1968–73.

[16] Bernardo C, Tavares A, Amaro T, Oliveira J, Afreixo V, Vale N, et al. Estrogen receptors expression in human bladder cancer and urogenital schistosomiasis. J Clin Oncol 35;15_suppl, doi:0.1200/JCO.2017.35.15_suppl.e16013 [accessed 9 November 2018]

[17] Backes FJ, Walker CJ, Goodfellow PJ, Hade EM, Agarwal G, Mutch D, et al. Estrogen receptor-alpha as a predictive biomarker in endometrioid endometrial cancer. Gynecol Oncol 2016;141:312–7:http://doi.org/0.1016/j.ygyno.2016.03.006.

[18] López-Romero R, Garrido-Guerrero E, Rangel-López A, Manuel-Apolinar L, Piña-Sánchez P, Lazos-Ochoa M, et al. The cervical malignant cells display a down regulation of ER-α but retain the ER-β expression. Int J Clin Exp Pathol 2013;6:1594–602.

[19] Mosny DS, Herholz J, Degen W, Bender HG. Immunohistochemical investigations of steroid receptors in normal and neoplastic squamous epithelium of the uterine cervix. Gynecol Oncol 1989;35:373–7.

[20] Monsonego J, Magdelenat H, Catalan F, Coscas Y, Zerat L, Sastre X. Estrogen and progesterone receptors in cervical human papillomavirus related lesions. Int J Cancer 1991;48:533–9.

[21] Nonogaki H, Fujii S, Konishi I, Nanbu Y, Ozaki S, Ishikawa Y, et al. Estrogen receptor localization in normal and neoplastic epithelium of the uterine cervix. Cancer 1990;66:2620–7.

[22] Kanai M, Shiozawa T, Xin L, Nikaido T, Fujii S. Immunohistochemical detection of sex steroid receptors, cyclins, and cyclin-dependent kinases in the normal and neoplastic squamous epithelia of the uterine cervix. Cancer 1998;82:1709–19.

[23] Stolnicu S, Barsan I, Hoang L, Patel P, Chiriboga L, Terinte C, et al. Diagnostic algorithmic proposal based on comprehensive immunohistochemical evaluation of 297 invasive endocervical adenocarcinomas. Am J Surg Pathol 2018;42:989–1000. https://doi.org/10.1097/PAS.0000000000001090.

[24] Shaw JL, Gislason GJ, Imbriglia JE. Transition of cystitis glandularis to primary adenocarcinoma of the bladder. J Urol 1958;79:815–22.

[25] Wiener DP, Koss LG, Sablay B, Freed SZ. The prevalence and significance of Brunn's nests, cystitis cystica, and squamous metaplasia in normal bladders. J Urol 1979;122:317–21.

[26] Andersen JA, Hansen BF. The incidence of cell nests, cystitis cystica, and cystitis glandularis in the lower urinary tract revealed by autopsies. J Urol 1972;108:421–4.

[27] Blakeman PJ, Hilton P, Bulmer JN. Estrogen and progesterone receptor expression in the female lower urinary tract, with reference to estrogen status. BJU Int 2000;86:32–8.

[28] Teng J, Wang ZY, Jarrard DF, Bjorling DE. Roles of estrogen receptor alpha and beta in modulating urothelial cell proliferation. Endocr Relat Cancer 2008;15:351–64. https://doi.org/10.1677/erc.1.01255.

[29] Hsu I, Yeh CR, Slavin S, Miyamoto H, Netto GJ, Tsai YC, et al. Estrogen receptor alpha prevents bladder cancer via INPP4B inhibited Akt pathway in vitro and in vivo. Oncotarget 2014;5:7917–35. https://doi.org/10.18632/oncotarget.1421.