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Original Article

Immunohistochemical analysis of sex hormone receptors in squamous changes of the urothelium

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Abstract: Objective: Squamous cell transformation of the urinary bladder urothelium has various causes, symptoms, and few treatment options. The aim of this study was to analyze and compare the expression of sex hormone receptors in non-keratinized and keratinized squamous metaplasia (NKSM, KSM), squamous cell carcinoma (SCC), and healthy urothelium with regard to possible therapeutic approaches. Methods: Biopsies from 26 patients with urothelial NKSM, KSM, and SCC were analyzed retrospectively. Tissue microarrays (TMA) of formalin-fixed paraffin-embedded (FFPE) bladder biopsies were stained with hematoxylin and eosin followed by immunohistochemical analysis with specific antibodies against estrogen, progesterone, and androgen receptors (ER, PR, AR) and assessment using the immunoreactive score. Statistical evaluations included the Wilcoxon signed-rank test and the Wilcoxon rank-sum test in the form of permutation tests. Results: Of the 15 women and 11 men included in this explorative study, 17 had metaplasia: 15 (six men, nine women) had NKSM and two KSM (both men). A total of nine patients (three men, six women) had keratinized SCC or urothelial carcinoma with squamous differentiation. The comparison between normal urothelial cells and metaplasia showed a significantly stronger expression in the metaplastic tissue (P=0.0374). The invasive carcinoma showed significantly less PR than the extracellular matrix of the healthy urothelium (P=0.0026). Expression of AR was nearly absent in healthy and metaplastic urothelium. Conclusion: There appears to be an association between squamous metaplasia of the bladder mucosa and sex steroid hormone receptor expression, especially estrogen receptors. Topical hormone therapy should be considered.

Keywords: Androgen receptor, estrogen receptor, progesterone receptor, squamous cell carcinoma, squamous metaplasia

Introduction

Squamous transformations of the bladder, such as non-keratinized squamous metaplasia (NKSM), keratinized squamous metaplasia (KSM) and squamous cell carcinoma (SCC), have a variety of causes, pathologic features, symptoms and therapeutic approaches. The different subtypes must be considered separately.

Much of the published literature to date has focused on NKSM which is a common occurrence in the female trigone [1]. In some patients, the finding of metaplasia in the trigone is incidental, without signs of inflammation and is also termed vaginal metaplasia [2-7]. Other patients demonstrate metaplasia of the trigone, together with cystoscopically or histologically visible signs of inflammation, which referred to as trigonitis. Symptoms include difficulties in bladder emptying, urge incontinence, suprapubic pain, and other lower urinary tract symptoms [2, 7, 8]. An influence of sex hormones on transformation of the urothelium to NKSM in the lower urinary tract is suspected [6, 8, 9]. Studies suggest that transformed urothelium of the bladder and the urethra is comparable to vaginal epithelium [7]. Influences such as age and physiological fluctuation of sex hormones in the menstrual cycle, pregnancy and menopause can influence these lesions [1]. However, others suggest that the metaplastic bladder epithelium is not subject to cyclic hormonal fluctuations, as is the endometrium, but is permanently susceptible to estrogen [9].

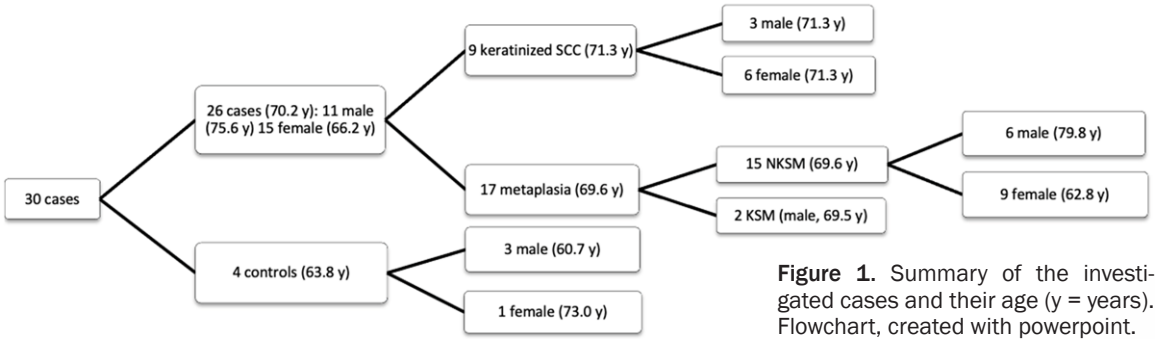

Figure 1. Summary of the investigated cases and their age (y = years). Flowchart, created with powerpoint.

Another possible cause of NKSM in the bladder appears to be recurrent infections [7, 10, 11]. Conversely, some authors suspect that squamous metaplasia is a predisposition for a bacterial reservoir and thus recurrent urinary tract infections occur in these patients [5, 12]. Bacteria can better adhere, multiply and remain in the altered epithelium with a disturbed glycosaminoglycan layer [6]. It is often difficult to effectively treat patients with recurrent urinary tract infections and bacteriologically permeable metaplastic epithelium by antibiotics. Bacterial resistance, allergy and costs are increasing [12]. Minimally invasive methods such as cystoscopic fulguration [6, 12] and Nd:YAG laser [11] seem to be promising therapies to remove squamous metaplasia in the urothelium.

Keratinized squamous metaplasia (KSM) mainly affects men [13] but is overall less common than NKSM. KSM can be caused by various factors such as urinary tract infections [14], bladder stones, parasites [15], vitamin A deficiency, radiation [1], fistulas, bladder exstrophy, obstruction [13] or indwelling catheterization [16]. These conditions can lead to permanent damage of the bladder mucosa and thus to KSM. In contrast to NKSM, carcinomas can develop from KSM [13]. The risk of malignancy increases with the duration and extent of transformation to KSM. Therefore, KSM should be removed as early and as completely as possible by suitable procedures [13, 14, 17, 18].

Squamous cell carcinoma (SCC) of the bladder is the second most common tumor of the urinary bladder, accounting for 2-5% of malignancies. SCC often occurs after recurrent inflammation or other chronic irritation especially after indwelling catheterization of the corresponding tissue. Smoking is another risk factor for SCC. Additionally the risk of SCC of the bladder specifically increases following Bilharzia infection and is higher in countries where schistosomiasis is endemic [19]. A link between the incidence of bladder cancer and sex steroid hormones is suspected and may lead to a more efficient target therapy [20-22]. Unfortunately, non-specific early symptoms of SCC often lead to late diagnosis and poor prognosis. The therapy of choice is radical cystectomy with lymph node resection [23].

The present exploratory study aims to compare the presence of steroid hormone receptors in squamous transformations with normal urothelium and possibly expand therapeutic options. It adds significantly to existing research by not only focusing on single disease, sex, bladder area (e.g. the trigone of the bladder) or steroid hormone receptors as previous studies have done, but instead identifying the presence of sex steroid receptors (ER, PR, AR) in non-keratinized and keratinized squamous transformations (metaplasia and carcinoma) of the urothelium.

Methods

Patients

Patients who underwent cystoscopy for various reasons and received a diagnosis of NKSM, KSM or SCC, were retrospectively included in this investigation. In total, tissue from 30 patients was examined. 26 of them had NKSM (n=15), KSM (n=2) metaplasia or SCC (n=9) of the urinary bladder (**Figure 1**). Urothelial carcinoma with squamous cell differentiation was counted as SCC. Two pathological and two normal samples could be obtained from most patients, allowing the normal ones to serve as internal controls. Four patients were found to

Table 1. Primary monoclonal antibodies and their characteristics

antibody against	clone	producer	buffer at 95°C	dilution	usage
ER	EP 1	Dako M3643	CC1 36 min.	01:40	32 min. at 37°C
PR	1 E 2	Ventana Roche790-2223	CC1 64 min.	-	10 min. at 36°C
AR	AR441	Dako M3562	CC1 36 min.	01:50	32 min. at 37°C

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; SD, standard deviation.

have inconspicuous urothelium and were used as controls. All biopsies were examined and archived as formalin fixed paraffin embedded (FFPE) tissues in the Institute of Pathology, Friedrich-Alexander-University Hospital Erlangen-Nürnberg (FAU) between 02/2015 and 12/2018.

Staining and immunohistochemistry

Tissue microarrays (TMA) were prepared from FFPE tissue blocks, creating a total of 94 samples examined on two slides. Hematoxylin-eosin staining and subsequent microscopy was used to confirm the specific pathology and severity of the inflammatory response. To determine the presence of sex hormone receptors semi-quantitatively, the samples were stained immunohistochemically with specific antibodies. First, the IHCH 1-2 µm thin paraffin sections of the TMA were incubated for 15 minutes at 80°C for deparaffinization to make the tissue sections stainable. Subsequently, tissues were immunohistochemically processed with Roche's fully automated slide staining system VENTANA BenchMark ULTRA® using specific primary antibodies for estrogen (Clone EP1, Dako), progesterone (Clone 1 E 2, Roche) and androgen (Clone AR441, Dako) receptors. Monoclonal antibodies and their properties are listed in **Table 1**.

The staining process was performed with Roche's VENTANA BenchMark ULTRA®. Prior to treatment, TMA sections were treated with the buffer Cell Conditioning 1 (CC1) and incubated at 95°C for epitope retrieval. The ultraView Universal DAB Detection Kit® from Ventana (760-500, Roche) was used for the subsequent reaction steps. In the first step, the endogenous peroxidase was inhibited by 3% hydrogen peroxide solution. The primary antibody mentioned above was then added in a specific dilution (**Table 1**). In the following steps a secondary antibody, a corresponding substrate, a chromogen, and copper were added for specific immunohistochemical staining. Finally, the sections were counterstained with hemalum. Samples with reliable expressions of estrogen, progesterone or androgen receptors were used as positive controls.

For ease of use and archiving, the slides equipped with TMA were scanned with the Digital Slide Scanner from Sysmex. Two different versions were used: Pannoramic 1000 (3DHistech) and Panoramic 250 (3DHistech). Slides were then digitally analyzed by two observers with the CaseViewer 2.3 (3DHistech, Budapest, Hungary).

The occurrence of each of these receptors was individually assessed in nuclei of metaplastic, cancerous, and normal tissue, and expression in epithelium and extracellular matrix was separately evaluated by two independent investigators using the immunoreactive score (IRS). The score is composed of the intensity of the staining (0= negative, 1= weak, 2= moderate, 3= strong) multiplied by the percentage of cells with nuclear positivity (0=0, 1<10%, 2=10-50%, 3=51-80%, 4>80%). The resulting outcomes are defined as follows: 0-2= negative; 4= weakly positive; 6, 8= moderately positive; 9, 12= strongly positive [24].

Statistics

Data were statistically processed with a proprietary program in programming language R. For quantitative traits, mean, median and the standard deviation were determined. In this context, for a more differentiated assessment of the number and intensity of receptor-positive cells, the IRS values 0-12 were preferred. To compare the occurrence of estrogen, progesterone and androgen receptors in squamous transformation compared to healthy tissue in the same person, the Wilcoxon signed-rank test was used. The Wilcoxon two-sample test (Mann-Whitney-U-test) was applied to compare metaplastic or tumorous samples with a

Table 2. All included patients with characteristics, urogenital symptoms, diagnoses, and pre-existing conditions

No	gender	age	tissue	inflam*	symptoms	diagnoses/pre-existing conditions
1	f	39	NKSM	++	dysuria, pollakisuria	rUTI, chronic pelvic pain syndrome
2	m	79	NKSM	+++	macrohaematuria	prostate Ca
3	f	83	NKSM	+++	urge incontinence, dysuria	rUTI
4	m	81	NKSM	++	macrohaematuria, urinary retention	BPH
5	m	77	NKSM	++	macrohaematuria, urinary retention	BPH-therapy with 5α-Reductase-inhibitor, condition after UC
6	m	57	KSM	++	unknown	condition after UC, epidymo-orchitis, nicotine abuse
7	f	42	NKSM	++	dysuria, pollakisuria	rUTI, chronic pelvic pain syndrome
8	m	79	NKSM	+	macrohaematuria	BPH, IDC, bladder stone
9	f	67	NKSM	+++	unknown	condition after nephrogenic adenoma
10	f	63	NKSM	++	unknown	cyst of bladder neck
11	f	70	NKSM	-	macrohaematuria, incontinece	condition after cervical Ca, hysterectomy
12	f	68	NKSM	-	unknown	condition after uterine Ca, radiotherapy, rUTI
13	m	76	NKSM	+++	macrohaematuria	condition after prostate Ca: radical prostatectomy, radiotherapy, antiandrogen therapy, IDC
14	m	82	KSM	++	macrohaematuria	condition after UC, chronic renal insufficiency
15	f	58	NKSM	+	dysuria	condition after hysterectomy, bladder stone, nicotine abuse
16	m	87	NKSM	-	macrohaematuria	condition after prostate Ca, radiotherapy, chronic renal insufficiency
17	f	75	NKSM	+++	unknown	unknown
18	m	80	SCC	++	nycturia	SCC pT2bN0(0/3)LoVoPn0G3
19	f	76	SCC	-	macrohaematuria, urinary retention	SCC trigonal, G3, IDC, UTI, condition after pancreas Ca
20	f	57	SCC	+	unknown	5×4 cm SCC posterior wall, UTI, condition after mamma Ca, nicotin abuse
21	f	76	SCC	+++	noctuia, dysuria, urinary retention	SCC posterior wall, condition after cervical Ca, nicotine abuse
22	f	76	SCC	+++	unknown	necrotizing SCC pT1L0V0G2 condition after metastatic renal cell Ca, hysterectomy
23	m	66	SCC	+++	macrohaematuria	SCC pT1L0V0G3, condition after prostatectomy
24	f	75	SCC	+++	unknown	SCC anterior wall, pT2G3, condition after bladder stones, chronic renal insufficiency
25	m	68	SCC	+++	unknown	SCC pT4pN1G3 with prostatic infiltration
26	f	68	SCC	+++	macrohaematuria	high grade SCC, pyelonephritis, dementia, condition after nicotine abuse

*Concomitant inflammatory reaction: +++ strong, ++ moderate, + week, - no. NKSM, non-keratinized squamous metaplasia; KSM, keratinized squamous metaplasia; SCC, squamous cell carcinoma; UC, urothelial carcinoma; rUTI, recurrent urinary tract infection; BPH, benign prostatic hyperplasia; IDC, indwelling urinary catheter; Ca, carcinoma.

healthy cohort and to contrast metaplastic tissue with carcinoma. The tests were carried out as permutation tests with 100,000 runs. The reasons for doing so were small sample sizes, the presence of ties in the data and the lack of convenient statistical distributions. No adjustment for multiple testing was performed due to low power from small numbers of individuals in the samples. Hence, caution had to be taken when interpreting *p*-values. Keeping this in mind, nevertheless, results were considered significant if $P<0.05$.

Results

Characteristics of the study population

In this study, 94 samples from 30 patients, who underwent cystoscopy with biopsies for various reasons, were examined. A total of 26 patients with different pre-existing conditions and symptoms had squamous transformations of the urothelium; NKSM (n=15), KSM (n=2) and SCC (n=9) of the bladder (**Table 2**). The median age was 70.2 years (male, n=11: 75.6 years, female

Table 3. Metaplasia compared to normal tissue

			mean	median	SD	*P*-value
ER	epithelium	metaplasia n=17	5.73	6	4.57	
		healthy tissue n=4	0	0	0	0.0374
	stroma	metaplasia n=17	1.35	1	1.5	
		healthy tissue n=4	2	1	2.89	0.908
PR	epithelium	metaplasia n=17	1.69	1	3.05	
		healthy tissue n=4	0.25	0	0.5	0.3816
	stroma	metaplasia n=17	3.94	4	2.86	
		healthy tissue n=4	3.5	4	1	0.7834
AR	epithelium	metaplasia n=17	0.44	0	0.73	
		healthy tissue n=4	0	0	0	0.5565
	stroma	metaplasia n=17	0.35	0	0.7	
		healthy tissue n=4	1	1	1.15	0.3118

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; SD, standard deviation.

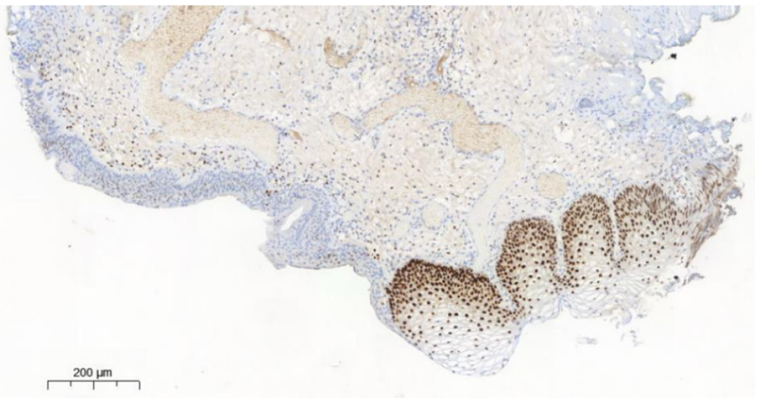

Figure 2. Immunohistochemical staining for ER. Low ER positivity in normal urothelium and subepithelial cells; high ER positivity in NKSM (×6). CaseViewer 2.3 (3DHistech, Budapest, Hungary).

n=15: 66.2 years). In 14 cases (10 metaplasia, 4 carcinoma), concomitant normal urothelium was available from the same biopsy procedure. In 12 of the 26 cases only pathological tissue could be obtained. In addition, eight samples from four patients with healthy urothelium served as controls (male, n=3: 60.7 years, female n=1: 73.0 years) among the internal physiological tissues. Samples that could not be assessed were excluded. Altogether 94 samples from 30 patients were included in the study (**Figure 1**).

Clinical outcome

Statistical analysis showed a significantly higher incidence of ER in squamous metaplasia compared to normal urothelium in healthy controls (P=0.0374) (**Table 3**). In most subjects, who had squamous metaplasia in a part of the bladder, no ER could be detected in their healthy urothelium (7/10) (**Figure 2**). Transitional urothelium was only clearly positive for ER in a few cases (3/10) (**Table 4**). The comparison between normal urothelium and metaplastic tissue of the same people demonstrated similar tendencies, with ER accumulating mainly in squamous metaplasia in the epithelium of women (7/9) and men (5/8) (**Table 4**). This did not reach statistical significance (P=0.0621). No relevant difference in ER expression was found in the underlying extracellular matrix with low expression of ER in sub-epithelial stroma detected in both cohorts (**Figure 2**).

The evaluation of SCC tissue in this study showed that this malignancy rarely develops sex hormone receptors. Compared to SCC tissue, PR was expressed more frequently in fibroblasts of healthy extracellular matrix (P=0.0026) (**Table 5**; **Figures 3** and **4**). The difference of PR in metaplastic and healthy stroma in the same person, though not statistically significant (P=0.0629), emphasizes the aforementioned result. PR was found primarily in basal cell layers of normal urothelium and in nuclei of fibroblasts in the underlying extracellular matrix. To a lesser extent PR occurred in squamous metaplasia or invasive carcinoma of the bladder epithelium.

The lack of sex hormone receptors in keratinized SCC was also evident when comparing the occurrence of those receptors in metaplasia and invasive carcinoma (**Table 6**). Differences became evident when comparing ER in epithelium (P=0.0079) and stroma (P=0.0366) and PR in epithelium (P=0.0464) and stroma (P=0.0003). In most cases, no normal epithelium

Table 4. ER in the epithelium in metaplasia and normal urothelium of the same individual

IRS-level: ER in epithelium												
No	gender	age	keratinization	localization	n.e.*	0	1	2	4	6	8	9	12
1	f	39	NKSM	fundus, trigonal		●						●	
2	m	79	NKSM	prostatic urethra	●					●			
3	f	83	NKSM	posterior & lateral wall		●	●						
4	m	81	NKSM	prostatic urethra	●							●	
5	m	77	NKSM	posterior wall			●						●
6	m	57	KSM	fundus, left lateral wall						●	●		
7	f	42	NKSM	fundus					●				●
8	m	79	NKSM	bladder neck	●	●							
9	f	67	NKSM	posterior wall	●	●							
10	f	63	NKSM	trigonal									● ●
11	f	70	NKSM	bladder, not specified		●			●				
12	f	68	NKSM	bladder, not specified		●		●					
13	m	76	NKSM	posterior wall	●			●					
14	m	82	KSM	posterior, left lat. wall, fundus		●	●						
15	f	58	NKSM	trigonal	●						●		
16	m	87	NKSM	right ostium, Papilloma	●	●							
17	f	75	NKSM	trigonal		●				●			

IRS, Immunoreactive score; *n.e., non-existent; NKSM, non-keratinized squamous metaplasia; KSM, keratinized squamous metaplasia; ● metaplasia; ● normal epithelium.

Table 5. Invasive carcinoma compared to normal tissue

			mean	median	SD	*P*-value
ER	epithelium	carcinoma n=9	0	0	0	
		healthy tissue n=4	0	0	0	1
	stroma	carcinoma n=9	0.22	0	0.67	
		healthy tissue n=4	2	1	2.83	0.2811
PR	epithelium	carcinoma n=9	0	0	0	
		healthy tissue n=4	0.25	0	0.5	0.7999
	stroma	carcinoma n=9	0.11	0	0.33	
		healthy tissue n=4	3.5	4	1	0.0026
AR	epithelium	carcinoma n=9	0	0	0	
		healthy tissue n=4	0	0	0	1
	stroma	carcinoma n=9	0	0	0	
		healthy tissue n=4	1	1	1.15	0.1523

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; SD, standard deviation.

could be identified in the carcinoma tissue biopsies, thus the number of samples was small and the resulting significance weak. Nevertheless, a comparison of the receptor expression in the extracellular matrix showed that almost no sex hormone receptors were found in SCC (**Figure 3**). This association emphasizes the high incidence of ER in the epithelium of squamous metaplasia and the lack of such receptors in SCC compared to healthy urothelium. It should also be noted that three of the nine patients with SCC were heavy smokers (**Table 2**).

Comparisons of the occurrence of AR in general did not show differences between squamous metaplasia or carcinoma and normal urothelium (**Tables 3**, **5**, **6**). Further, comparisons of sex steroid receptors in pathologic and healthy tissues of the same individual did not yield result of different expression patterns.

Additionally, a mild to severe inflammatory reaction with leukocyte infiltration and edema has been noted in the subepithelial stroma of squamous pathologies in most patients (**Figure 5**; **Table 2**).

Discussion

This study was the first to date to evaluate ER, PR, and AR occurrence in NKSM, KSM and SCC in female and male urothelium. Biopsies were

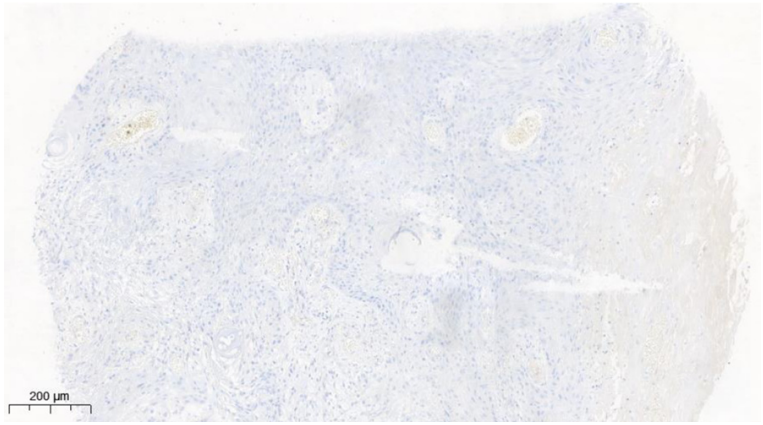

Figure 3. Immunohistochemical staining for PR. Negative staining for PR in SCC (×6). CaseViewer 2.3 (3DHistech, Budapest, Hungary).

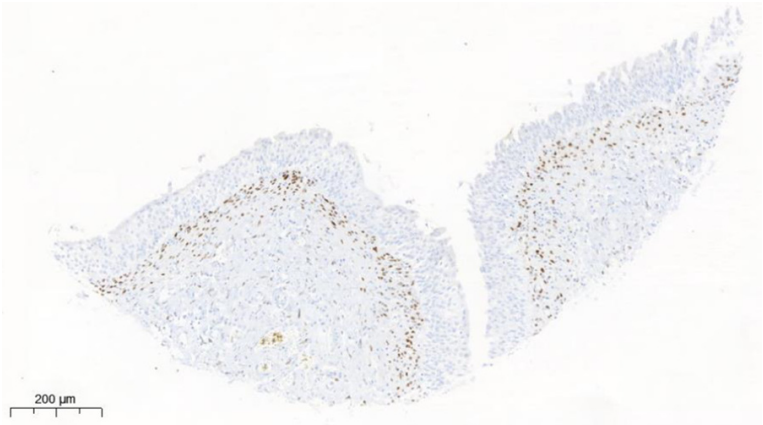

Figure 4. Immunohistochemical staining for PR. Positive staining for PR in sub-epithelial cells in normal urothelium (×5). CaseViewer 2.3 (3DHistech, Budapest, Hungary).

Table 6. Metaplasia compared to invasive carcinoma

			mean	median	SD	*P*-value
ER	epithelium	metaplasia n=17	5.73	6	4.57	
		carcinoma n=9	0	0	0	0.0079
	stroma	metaplasia n=17	1.35	1	1.5	
		carcinoma n=9	0.22	0	0.67	0.0366
PR	epithelium	metaplasia n=17	1.69	1	3.05	
		carcinoma n=9	0	0	0	0.0464
	stroma	metaplasia n=17	3.94	4	2.86	
		carcinoma n=9	0.11	0	0.33	0.0003
AR	epithelium	metaplasia n=17	0.44	0	0.73	
		carcinoma n=9	0	0	0	0.3334
	stroma	metaplasia n=17	0.35	0	0.7	
		carcinoma n=9	0	0	0	0.3201

ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; SD, standard deviation.

taken from selected regions of the bladder wall that appeared suspicious to the examining urologist. Interestingly, we found that NKSM occurred not only in the trigone of the bladder in women, as described in the literature, but also affected other areas of the bladder and occurred in male patients.

The literature on urothelial metaplasia to date has focused on NKSM in the female trigone of the bladder [1-5, 8, 9, 11, 12]. Our study shows that the incidence of ER is significantly higher in squamous metaplasia than in healthy urothelium. In almost half of the women examined in this retrospective study, the NKSM was located in the vesical trigone and showed a moderate to strong incidence and intensity of ER. However, other areas of the bladder were also affected by this pathology and expressed ER. Other studies have detected ER in NKSM by immunohistochemistry in female bladders [3, 8, 9] and pointed to an influence of estrogen on metaplasia [8, 9, 25]. However, the exact reason why ER expression is higher in squamous metaplasia of the bladder than in normal urothelium is not yet known. One possible explanation could be the lack of estrogens. Many postmenopausal women suffer from estrogen deficiency which may lead to a higher likelihood of developing recurrent urinary tract infections [26]. It is possible that the urothelium forms squamous metaplasia with increased ER under estrogen deficiency. The squamous epithelium is more susceptible to bacterial adherence and invasion [6], resulting in recurrent urinary tract infections that

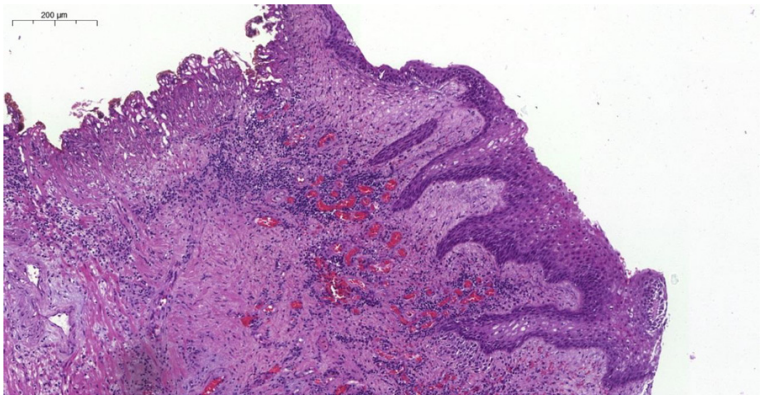

Figure 5. H&E staining. Sub-epithelial inflammation in squamous metaplasia (×7). CaseViewer 2.3 (3DHistech, Budapest, Hungary).

are difficult to treat with antibiotics [12]. We also found higher inflammatory activity with leukocyte infiltration and edema in almost all of the metaplastic tissues examined, but not in the control tissue studied. The results of our study in addition with previous literature, suggest that in women with ER-positive NKSM and recurrent urinary tract infections, topical e.g. vaginal estrogen therapy might relieve symptoms [27]. Conversely, the small group of patients with ER-negative NKSM must be considered. In these patients, an irritative cause is suspected [3], in which hormonal therapy would probably not be effective. These associations need to be verified in a prospective study in the near future. It is important to distinguish between ER-positive and ER-negative NKSM with regard to cause, symptom expression and therapy options.

Since PR occurs mainly in basal cells of healthy female urothelium, Pacchioni et al. suggested that progesterone is important for normal bladder mucosa and vaginal application could lead to alleviation of pseudomembranous trigonitis [3]. Another study found that the expression of PR in the urogenital area was dependent on serum estrogen levels and higher in the urogenital tract in premenopausal women [9]. In this study, PR was observed in cell nuclei of the basal layers of healthy and metaplastic urothelium and in fibroblasts in both sexes. More detailed research with a larger number of subjects is necessary to make concrete statements.

In the present study, AR was near absent regardless of tissue type and sex. AR has only very rarely been discussed in the previously published literature on sex hormone receptors in metaplastic urothelium. The literature focuses mainly on the relationship between androgen and bladder cancer in general [20, 22, 28] or urothelial carcinoma of the bladder (UC) [21, 29]. Since bladder cancer is three times more common in men than in women [30], the cause for which is still unknown, different sex hormone pathways may be implicated leading to discussion of hormonal therapeutic approaches [20, 21, 29]. For example, some studies demonstrate AR expression in most superficial carcinoma but not in invasive carcinoma [21, 22]. Therefore, the possibility of antiandrogenic therapy for superficial UC can be discussed [21]. Another study with 472 patients concluded that AR is infrequently expressed in tumor cells of urothelial carcinoma regardless of gender or tumor grading/staging [29]. ERα and PR also do not occur in the UC, according to another study [31]. Our study shows that invasive SCC and urothelial carcinoma with squamous differentiation of the bladder infrequently develop any sex hormone receptors. Therefore, we suspect that anti-hormonal therapy would not be effective for the treatment of SCC, but further studies with a larger study population are urgently needed for this type of cancer to verify these considerations.

As can be seen from this work, there are very few clinical studies that have investigated the sex hormone receptors ER, PR, and AR in metaplastic pathologies of the urothelium in female and male patients. An acknowledged limitation of the retrospective data is the heterogeneity of the small patient population. For example, tissues from only two patients with KSM could be studied. This limits the conclusions that can be drawn. Prospective studies with larger cohorts of men and women of all ages are urgently needed to investigate the different pathological subtypes for the presence of sex hormone receptors. This may lead to new clinical and therapeutic possibilities in the future.

Conclusion

This exploratory study investigated sex hormone receptors ER, PR and AR in NKSM, KSM

and SCC of the urothelium compared with normal urothelium. We found that ER was more abundant in the epithelium of squamous metaplasia in female and male patients. ER-positive squamous metaplasia has been found not only in the trigone of women but affects other areas of the bladder and also occurs in men. A relationship between sex steroid hormones and NKSM and KSM of the urinary bladder is probable, leading to the suggestion that hormonal therapeutic approaches may provide relief. By contrast, SCC expressed a paucity of sex steroid receptors and is unlikely to respond to hormonal therapy.

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Disclosure of conflict of interest

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