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ORIGINAL ARTICLE

The Importance of Expression of Keratin 17 (KRT17) and SPINK1 in Neoplastic (Invasive and Noninvasive) Lesions of the Bladder

Mesanenin Neoplastik (İnvaziv ve Noninvaziv) Lezyonlarında Keratin 17 (KRT17) ve SPINK1 Ekspresyonunun Önemi

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ABSTRACT

Objective: Diagnosis and grading of bladder cancers have a significant impact on treatment and prognosis. However, there are currently no very sensitive and specific immunohistochemical panels that can be used in the differential diagnosis of neoplastic lesions of the bladder and histomorphological findings are still the gold standard. We aimed to define the potential diagnostic importance of SPINK1, KRT17 and Laminin immunostaining in distinguishing neoplastic bladder lesions.
Material and Methods: KRT17, SPINK1 and Laminin expressions were assayed in 141 tissue samples of non-neoplastic bladder mucosa (NBM) and neoplastic bladder lesions by immunohistochemistry.
Results: KRT17 and SPINK1 are often expressed in tumoral tissues (86.2% and 68.7%, respectively). A statistically significant difference in KRT17 immunostaining was detected between the NBM and all seven neoplastic groups (p=0.03 to p□0.001). SPINK1 expression was significantly lower in NBM in comparison to neoplasms. Staining 2.5% or more of cells in tumor tissue with KRT17 distinguishes neoplastic lesions from nonneoplastic lesions with a sensitivity of 86.3% and a specificity of 100%. However, staining 12.5% or more of the cells in the tumor tissue with SPINK1 distinguishes neoplastic lesions from nonneoplastic lesions with a sensitivity of 62.6% and a specificity of 60%. Although both KRT17 and SPINK1 were stained in 60% of neoplasms, neither KRT17 nor SPINK1 staining was seen in 5.3% of neoplastic patients.
Conclusion: Immunohistochemical panel consisting of KRT17, SPINK1 and Laminin can be used together with morphological findings for accurate diagnosis of bladder neoplasia.

Keywords: Bladder, Cancer, Immunohistochemistry, KRT17, SPINK1, TAT1

ÖZ

Amaç: Mesane kanserlerinin tanı ve derecelendirilmesi tedavi ve prognoz üzerinde önemli bir etkiye sahiptir. Ancak günümüzde mesanenin neoplastik lezyonlarının ayırıcı tanısında kullanılabilecek çok hassas ve spesifik immünohistokimyasal paneller yoktur ve histomorfolojik bulgular halen altın standart olarak kabul edilmektedir. SPINK1, KRT17 ve Laminin immün boyalarının neoplastik mesane lezyonlarını ayırt etmedeki potansiyel önemini göstermeyi amaçladık.
Gereç ve Yöntem: KRT17, SPINK1 ve Laminin ekspresyonları immünohistokimyal yöntemle, toplam 141 doku örneğinde, neoplastik olmayan mesane mukozası (NBM) ve neoplastik mesane lezyonlarında araştırıldı.
Bulgular: KRT17 ve SPINK1 sıklıkla tümör dokularında eksprese edilir (sırasıyla %86,2 ve %68,7). NBM ve yedi neoplastik grubun tümü arasında KRT17 immün boyamasında istatistiksel olarak anlamlı bir fark tespit edildi (p=0.03 ila p□0.001). NBM'de SPINK1 ekspresyonu, neoplazmlara kıyasla önemli ölçüde daha düşüktü. KRT17 ile tümör dokusundaki hücrelerin %2,5 veya daha fazlasının boyanması, neoplastik lezyonları neoplastik olmayan lezyonlardan, %86,3 duyarlılık ve %100 özgüllük ile ayırır. Bununla birlikte, tümör dokusundaki hücrelerin %12,5 veya daha fazlasının SPINK1 ile boyanması, neoplastik lezyonları neoplastik olmayan lezyonlardan, %62.6 duyarlılık ve %60 özgüllük ile ayırır. Neoplazmların %60'ında hem KRT17 hem de SPINK1 boyanmış olmasına rağmen, neoplastik hastaların %5,3'ünde ne KRT17 ne de SPINK1 boyanması görülmedi.
Sonuç: KRT17, SPINK1 ve Laminin'den oluşan immünohistokimyasal panel, mesane neoplazisinin doğru tanısında morfolojik bulgularla birlikte kullanılabilir.

Anahtar Kelimeler: Mesane, Kanser, İmmünohistokimya, KRT17, SPINK1, TAT1

Introduction

Bladder cancer is the 12th most common type of cancer in the world. More than 90% of bladder cancers are urothelial carcinomas. Although non-muscle invasive carcinomas frequently recur, they have a better prognosis, while muscle invasive ones have a worse prognosis (1). Accuracy of grade and stage of urothelial tumors are very important factors in terms of risk classification and management (2). The recurrence and stage progression rates in Papillary urothelial neoplasm of low malignant potential (PUNLMPs) are 36% and 4%, respectively, while they are 50% and 10% in low-grade carcinomas (3). In high-grade carcinomas, the recurrence rate is 60%, the lamina propria invasion rate is 25%, and the muscularis propria invasion rate is 5% (4).

Despite the potential utility of the IHC panel of CK20, P53 and CD44 in differentiating reactive atypia from carcinoma in situ according to ISUP, morphology remains the gold standard and IHC does not have a significant role in grading papillary urothelial carcinomas and distinguishing between dysplasia and carcinoma in situ (5).

It is not always easy to differentiate basing on morphological findings alone low grade tumoral lesions from nonneoplastic lesions and low grade carcinomas in bladder. Therefore, there is currently a need for immunohistochemical antibodies and panels to enable this distinction.

Serine peptidase inhibitor Kazal type 1 (SPINK1) also known as tumor-associated trypsin inhibitor (TATI) and pancreatic secretory trypsin inhibitor (PSTI) are protease inhibitors. These protease inhibitor play a role in cell proliferation, cancer pathogenesis and inflammation (6) and have also been demonstrated in urothelial cells (7). KRT17 is expressed in basal cells of the epithelium and is also involved in cell proliferation and growth (8).

Today, the lack of immunohistochemical antibodies and panels that will enable this distinction to be made definitively leads to the need for them. To contribute to this distinction, we investigated the expressions of both KRT17, SPINK1 and laminin in bladder lesions. Although there has been only one study on the discriminatory value of KRT17 in bladder lesions in the literature[8], SPINK1 has not been studied previously in this context.

Materials and Methods

1.Sample selection

This study included a total of 141 FFPE (Formalin-Fixed Paraffin-Embedded) tissue blocks (131 bladder tumors and 10 non-neoplastic bladder mucosa "NBM") from 141 individual randomly selected patients, obtained between 2010 and 2018. These specimens were obtained during biopsies and during TUR (transurethral resection) of the bladder tumours from 141 patients of 123 whom were male with a mean age of 66 years (range 44-90 years) and 18 females with a mean age of 73 years (range 53-91 years).

These patients included NBM (n = 10), PUNLMP, (n=20), non-invasive papillary urothelial carcinoma-low-grade (NIPUC-LG, n=20), infiltrating urothelial carcinoma-low grade (IUC-LG, n=15), urothelial carcinoma in situ (UCIS, n=18), non-invasive papillary urothelial carcinoma-high-grade (NIPUC-HG, n=18), infiltrating urothelial carcinoma, high-grade, non-muscle-invasive (IUC-HG-NMI, n=20) and infiltrating urothelial carcinoma, high-grade, muscle-invasive (IUC-HG-MI, n=20). All the patients were assessed retrospectively by two independent pathologists (SU and IH) and reported as per the WHO/ISUP Classification 2016.

Selcuk University Faculty of Medicine Institutional Ethics Committee approved this study. (February 06, 2019, number: 2019/66).

2. Immunohistochemistry (IHC)

Immunohistochemistry was performed with the Ventana Benchmark XT and DAKO Omnis autostainer.

KRT17, SPINK1, CD31 and D2-40: Sections of 3-4 µm thickness from FFPE tissue blocks from each patient were taken, then deparaffinized and rehydrated. After heat induced antigen retrieval with EDTA buffer (pH 8.0), endogenous peroxidase was quenched with 3% hydrogen peroxide for 15 minutes. After monoclonal mouse anti-human antibodies for KRT17 (Clone E3, Ready to Use, DAKO), SPINK1 (Clone 4D4, Dilution 1/500, Novus Biologicals), CD31 (Clone JC70A, Ready to Use, DAKO), and D2-40 (Clone D2- 40, Ready to Use, DAKO) was performed, diaminobenzidine was used for 3 minutes. Hematoxylin-eosin dye was administered for counterstaining.

Positive control tissues were epidermoid carcinoma, normal pancreas and normal appendix for KRT17, SPINK1, and CD31 and D2-40, respectively. Positive controls were included in every batch of IHC staining. For lymphovascular invasion, CD31 and D2-40 antibodies were used.

Laminin: Sections of 3-4 µm thickness from FFPE tissue blocks from each patient were taken, then deparaffinized and rehydrated. After heat induced antigen retrieval with protease, endogenous peroxidase was quenched with 3% hydrogen peroxide for 15 minutes. Then monoclonal, Mouse anti-human antibody for Laminin (Clone 4C7, Dilution 1/20, DAKO) was performed, and diaminobenzidine was used for 3 minutes. Hematoxylin-eosin dye was administered for counterstaining.

Positive control tissue for Laminin was the renal cortex. Positive controls were included in every batch of IHC staining. To investigate the presence of invasion, the anti-laminin antibodies were applied.

3. Interpretation of Immunostaining Results

IHC results were determined using a semi-quantitative visual approach after immunoreactivity on each slide was evaluated by two experienced pathologists (SU and IH).

The scoring for SPINK1 and KRT17 immunostaining were done manually. Cytoplasmic SPINK1 and KRT17 immunopositivity were evaluated by the proportion of positively stained cells.

The proportion of SPINK1 and KRT17 positive cells was evaluated as a percentage. Absence of positivity was scored as 0.

The staining intensity of SPINK1 and KRT17 positive cells was grouped as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining).

KRT17 was considered positive regardless of the proportion and intensity, since it was not stained in the normal urothelium. For statistical analysis, the patients were divided into 2 groups as negative (score 0) and positive (score 1).

SPINK1 was considered positive with a score of 2 at a minimum of 10% of tumor cells, since it was often (88%) scored 1 or 0 (negative) in all cell layers of the normal urothelium except for umbrella cells in the NBM.

Umbrella cells were stained 3 positive in all normal control tissues. For statistical analysis, the patients were divided into 2 groups as negative (score 0) and positive (score 1).

4. Statistical analysis

The expressions of each KRT17 and SPINK1 protein were statistically analyzed using the SPSS Version 18.0. Kolmogrov-Smirnov and Shapiro-Wilk test was used to assess the distribution of the data. Kruskal-Wallis test was used to find out whether there was a statistically significant difference between all groups.

In comparisons of more than 2 groups, the homogeneity of variances was evaluated with the Levene test. Since the variances are not distributed homogeneously, Tamhane's T2 was used as Post-Hoc correction in binary comparisons to find the source of the difference.

Difference in expression of KRT17 and SPINK1 proteins between high-grade and low-grade bladder tumor samples, between non-invasive and invasive bladder tumor samples compared to NBM was assessed with Kruskal-Wallis test. When a difference was found, Tamhane's T2 was used as a Post-Hoc correction in binary comparisons to find the source of the difference. Mann Whitney U test was used because our data did not demonstrate normal distribution. Chi-square test was performed to compare the expression frequency rates of KRT17 and SPINK1 in neoplasia and NBM. Receiver operating curves analyses were used to compare KRT17 and SPINK1 expression in NBM and neoplastic tissues. ROC and the area under the curve were calculated to discriminate different diagnostic categories. Sensitivity and specificity were calculated, corresponding to the optimal cut-off values. $p < 0.05$ was considered statistically significant.

Results

1.Expression of KRT17, SPINK1 and Laminin in normal bladder mucosa

KRT17: Full-thickness staining of KRT17 was not detected in urothelial cells in NBM (Fig. 1-A).

Although focal strong KRT17 staining in basal urothelial cells in two NBMs and strong KRT17 staining in more than 50% of basal urothelial cells in one NBM, KRT17 staining was not detected in basal urothelial cells in the remaining 7 NBMs. Squamous metaplasia foci in NBM were not stained with KRT17. Hyperplastic and dysplastic urothelium showed weak staining for KRT17.

SPINK1: Umbrella cells showed strong staining for SPINK1 in all patients of NBM (Fig. 1-D). In three patients, no staining was observed in cells other than umbrella cells. The remaining seven NBM biopsies, SPINK1 staining of varying degrees and intensity was observed in urothelial cells other than umbrella cells.

Squamous metaplasia foci in NBM were not stained with SPINK1. Hyperplastic and dysplastic urothelium showed strong staining for SPINK1.

Laminin: The basement membrane underlying the unremarkable urothelium, the muscularis mucosae, the detrusor muscle and blood vessels in normal bladder were stained with Laminin.

2.Expression of KRT17, SPINK1 and Laminin in neoplastic bladder

KRT17 in neoplastic bladder

Statistically significant differences were observed in KRT17 staining between NBM and all bladder neoplasm groups (Table 1, Table 2 and Table 3), (Fig. 1A-C).
KRT17 positivity by staining proportion only, is an important diagnostic factor in distinguishing neoplastic bladder lesions and discriminating them from NBM ($p<0.001$), area under the curve: 0.93 (% 95 CI: 0.88-0.97). A cut-off of ≥2.5 for percentage of cells positively stained for KRT17, displayed a sensitivity of 86.3% and a specificity of 100% at the distinction neoplastic lesions from normal bladder mucosa (Fig. 2). When KRT17 was considered positive regardless of proportion and density, the sensitivity of KRT17 was 86.2 % and the specificity was 100 % in distinguishing neoplastic lesions from normal bladder mucosa.

Table 1: Comparison between NBM and all tumoral lesion groups regarding KRT17 expression

					95% Confidence Interval	
Group (n)	Group (n)	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NBM (10)	PUNLMP (20)	-76.750	6.532	0.000	-100.39	-53.11
	NIPUC-LG (20)	-69.500	7.163	0.000	-95.42	-43.58
	IUC-LG (15)	-42.333	10.338	0.030	-81.95	-2.71
	NIPUC-HG (18)	-49.444	9.375	0.002	-84.01	-14.88
	UCIS (18)	-28.333	6.919	0.021	-53.84	-2.82
	IUC-HG-NMI (20)	-32.000	6.943	0.005	-57.12	-6.88
	IUC-HG-MI (20)	-37.500	6.684	0.001	-61.68	-13.32

NBM: nonneoplastic bladder mucosa, PUNLMP: Papillary urothelial neoplasm of low malignant potential, NIPUC-LG: Non-invasive papillary urothelial carcinoma-low grade, IUC-LG: Infiltrating urothelial carcinoma-low grade, NIPUC-HG: Non-invasive papillary urothelial carcinoma, high-grade, UCIS: Urothelial carcinoma in situ, IUC-HG-NMI: Infiltrating urothelial carcinoma, high-grade, non-muscle-invasive, IUC-HG-MI: Infiltrating urothelial carcinoma, high-grade, muscle-invasive.

SPINK1 in neoplastic bladder

NBM had significantly lower expression of SPINK1 than PUNLMP, NIPUC-LG, LGN, HGN and NIN (Noninvasive Neoplasm) (Fig. 1D,3A,3B). All other findings are in Table 1, Table 2 and Table 3.
SPINK1 positivity according to staining proportion alone is an important diagnostic factor in distinguishing neoplastic bladder lesions and at the distinction them from NBM ($p=0.033$), area under the curve: 0.70 (% 95 CI: 0.58-0.82). A cut-off of ≥12.5 as the percentage of cells staining positive for SPINK1 displayed a

sensitivity of 62.6% and a specificity of 60% at the distinction neoplastic lesions from NBM (Fig. 4). SPINK1 immunopositivity score of 2 at a minimum of 10% of tumor cells, demonstrated a sensitivity of 68.7 % and a specificity of 50 % in discriminating neoplastic lesions from normal bladder mucosa.

KRT17 and SPINK1 are often expressed in tumoral tissues, (86.2% and 68.7%, respectively). However, there was no statistically significant difference between KRT17 and SPINK1 in terms of detecting neoplasia (p=0.455). Although both KRT17 and SPINK1 were stained in 60% of neoplasms, neither of them were stained in 5.3% of neoplasms (Table 4).

Laminin in neoplastic bladder

Staining with laminin was observed on basement membranes of all normal bladder and all non-invasive tumors (Fig. 3C). Laminin staining was also seen around the nests of carcinoma cells in 13 of 20 patients in the IUC-HG-MI group (Fig. 3D). CD31 and D2-40 were negative in these 13 patients. Staining with laminin was not observed in other invasive tumors and tumor cells.

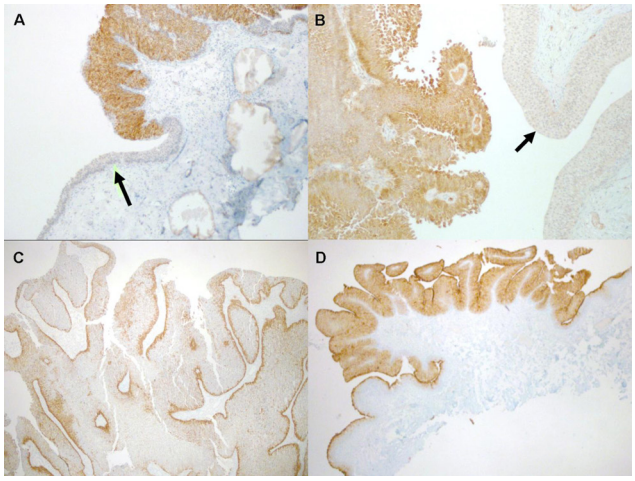

Figure 1: KRT17 and SPINK1 staining in NBM and bladder tumors

A: Strong KRT17 staining in PUNLMP, the normal urothelial cells were negative (arrow) (KRT17, original magnification, X 40)

B: Diffuse KRT17 staining in NIPUC-LG in left side, the normal urothelial cells were negative (arrow) (KRT17 stain, original magnification, X 100)

C: Low KRT17 expression in NIPUC-HG (KRT17 stain, original magnification, X 400)

D: Diffuse SPINK1 staining in PUNLMP and SPINK1 staining only in the umbrella cells in normal urothelial cells (in left side and right side) (SPINK1 stain, original magnification, X 40)

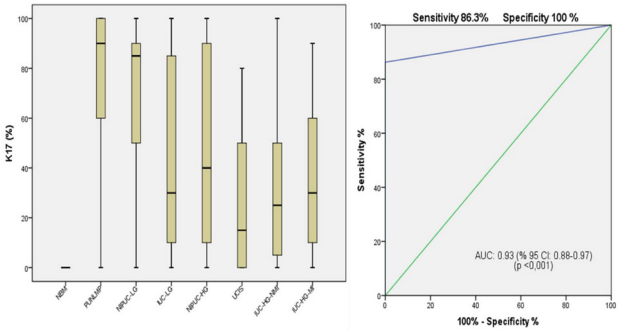

Figure 2: KRT17 expression by according to staining proportion in NBM and bladder tumors.

NBM: Nonneoplastic bladder mucosa, PUNLMP: Papillary urothelial neoplasm of low malignant potential, NIPUC-LG: Non-invasive papillary urothelial carcinoma, low-grade, IUC-LG: Infiltrating urothelial carcinoma-low grade, NIPUC-HG: Non-invasive papillary urothelial carcinoma, high-grade, UCIS: Urothelial carcinoma in situ, IUC-HG-NMI: Infiltrating urothelial carcinoma, high-grade, non-muscle-invasive, IUC-HG-MI: Infiltrating urothelial carcinoma, high-grade, muscle-invasive.

AUC Area under the curve, CI Confidence Interval.

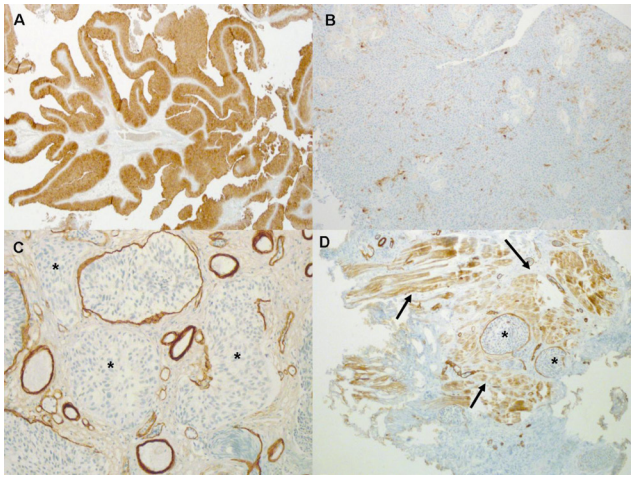

Figure 3: SPINK1 and Laminin expressions in NBM and bladder tumors

A: Diffuse and strong SPINK1 staining in NIPUC-LG (SPINK1 stain, original magnification, X 25)

B: Low SPINK1 expression in IUC-HG-NMI (SPINK1 stain, original magnification, X 100)

C: Although laminin is seen around the blood vessels and non-invasive tumor focus, there is no staining around the aggregates of invasive tumor cells (three asteriks) in the in IUC-HG-NMI group (Laminin stain, original magnification, X 200)

D: Laminin is seen in two invasive tumor focus (asteriks) and around the detrusor muscle (three arrows) in IUC-HG-MI group (Laminin stain, original magnification, X 100)

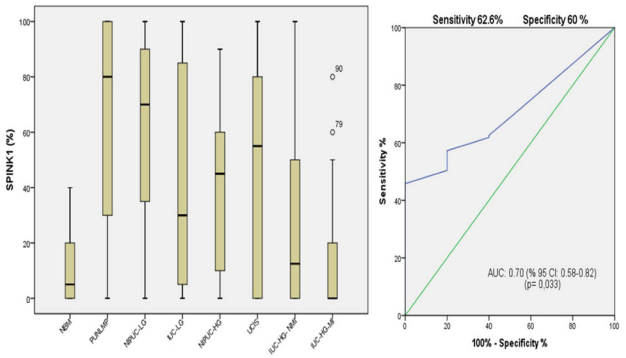

Figure 4: SPINK1 expression by according to staining proportion in NBM and bladder tumors.

NBM: Nonneoplastic bladder mucosa, PUNLMP: Papillary urothelial neoplasm of low malignant potential, NIPUC-LG: Non-invasive papillary urothelial carcinoma, low-grade, IUC-LG: Infiltrating urothelial carcinoma-low grade, NIPUC-HG: Non-invasive papillary urothelial carcinoma, high-grade, UCIS: Urothelial carcinoma in situ, IUC-HG-NMI: Infiltrating urothelial carcinoma, high-grade, non-muscle-invasive, IUC-HG-MI: Infiltrating urothelial carcinoma, high-grade, muscle-invasive.

AUC Area under the curve, CI Confidence Interval.

Table 2: Comparison Between KRT17 and SPINK1 expression in NBM and some tumoral lesions

Groups		KRT17			SPINK1		
		MD	P value	95% CI	MD	P value	95% CI
NBM	Noninvasive Neoplasm	-56.908	0.000	-67.34 -46.48	-39.500	0.000	-56.74 -22.26
	Infitrating Neoplasm	-36.818	0.000	-47.77 -25.87	-14.545	0.135	-32.33 3.24
Noninvasive Neoplasm	Infitrating Neoplasm	20.090	0.004	5.17 35.01	24.955	0.000	9.55 40.36
NBM	Low Grade Neoplasm	-64.727	0.000	-76.61 -52.85	-43.000	0.000	-61.55 -24.45
	High Grade Neoplasm	-36.711	0.000	-45.94 -27.48	-18.908	0.025	-35.79 -2.02
Low Grade Neoplasm	High Grade Neoplasm	28.017	0.000	13.16 42.87	24.092	0.001	8.15 40.04

MD: Mean Difference, CI: Confidence Interval, NBM: Nonneoplastic bladder mucosa (n=10), Noninvasive Neoplasm (n=76), Infitrating Neoplasm (n=55), Low Grade Neoplasm (n=55), High Grade Neoplasm (n=76)

Table 3: Comparison of KRT17 and SPINK1 expressions according to staining proportion in NBM and neoplastic lesions

	SPINK1			KRT17		
Groups	Median	Min	Max	Median	Min	Max
NBM	5	0	40	0	0	0
N	40	0	100	50	0	100
NIN	60	0	100	70	0	100
IN	10	0	100	30	0	100
LGN	60	0	100	80	0	100
HGN	20	0	100	30	0	100
PUNLMP	80	0	100	90	0	100
NIPUC-LG	70	0	100	85	0	100
IUC-LG	30	0	100	30	0	100
UCIS	55	0	100	15	0	80
NIPUC-HG	45	0	90	40	0	100
IUC-HG-NMI	12,5	0	100	25	0	100
IUC-HG-MI	0	0	80	30	0	90

NBM: nonneoplastic bladder mucosa, N: Neoplasm, NIN: Noninvasive Neoplasm, IN: Infiltrating Neoplasm, LGN: Low Grade Neoplasm, HGN: High Grade Neoplasm, PUNLMP: Papillary urothelial neoplasm of low malignant potential, NIPUC-LG: Non-invasive papillary urothelial carcinoma, low-grade, IUC-LG: Infiltrating urothelial carcinoma-low grade, UCIS: Urothelial carcinoma in situ, NIPUC-HG: Non-invasive papillary urothelial carcinoma, high-grade, IUC-HG-NMI: Infiltrating urothelial carcinoma, high-grade, non-muscle-invasive, IUC-HG-MI: Infiltrating urothelial carcinoma, high-grade, muscle-invasive.

Table 4: Comparison of KRT17 and SPINK1 expressions in NBM and neoplasms

	NBM (n=10)	Neoplasm (n=131)
KRT17	Negative (100 %)	113 positive (86.2 %) 18 negative (13.8 %)
SPINK1	5 positive (50 %) 5 negative (50 %)	90 positive (68.7 %) 41 negative (31.3 %)

NBM: Nonneoplastic bladder mucosa

Discussion

Today, the importance of immunohistochemical methods in the definitive diagnosis dysplasia and carcinoma in situ and grading of urothelial carcinomas of the bladder are very limited. Because cross-reactions, aberrant expressions or loss of expression can be seen in antibodies and/or antibodies used for this purpose. As a result, a definitive diagnosis cannot always be made with these antibodies. This leads to more reliance on morphological findings.

KRT17 is expressed in carcinomas of the breast, cervix, and stomach, as well as epidermoid carcinomas and adenocarcinomas, and KRT17 levels are also associated with metastasis. These show that KRT17 may have diagnostic and prognostic significance in cancers (9).

KRT17 promoting cell proliferation and migration is persistently induced in oral epidemoid carcinoma (10). Dasgupta et al suggested that increased KRT17 expression supported the diagnosis of differentiated vulvar intraepithelial neoplasia (11). Immunohistochemically, KRT17 expresses more prominently in epidermoid carcinomas of the cervix and HSILs (=high-grade squamous intraepithelial lesions) than in normal squamous mucosa and LSILs (=low-grade squamous intraepithelial lesions). Therefore, KRT17 can be used to diagnose epidermoid carcinomas and HSILs of the cervix (12). High expression of KRT17 in in situ endocervical adenocarcinoma and invasive endocervical adenocarcinoma adversely affects survival (13).

Fernandez-Flores et al. suggest that KRT17 expression shows atypical foci in actinic keratosis or Bowen disease (14). KRT17 is markedly expressed and indicates poor survival in estrogen receptor-negative and human epidermal growth factor receptor-2-negative breast cancers (15).

The present study showed that when KRT17 was considered positive regardless of proportion and density, KRT17 expression was significantly increased across all categories of neoplasms compared with NBM. In addition, our study demonstrated that there was a significant increase in KRT17 positivity of tumor cells according to staining proportion alone in all neoplasm groups when compared to normal urothelial mucosa. These observations are important in distinguishing normal urothelial mucosa and neoplasms especially in small biopsy specimens.

We found that KRT17 expression was most prominent in PUNLMP. KRT17 expression was higher in low grade neoplasms than in high grade neoplasms ($p<0.001$) and KRT17 expression was higher in NIN than infitrating Neoplasms ($p=0.004$). The present study revealed that there was no statistically significant difference between IUC-HG-NMI and IUC-HG-MI ($p=1.000$) in terms of KRT17 expression.

These findings indicate that KRT17 demonstrates lower immunopositivity in infiltrating and high grade urothelial tumors compared to NIN and low grade urothelial tumors. This could be explained by the biological behavior of the tumors.

Babu et al. found that KRT17 was a sensitive and specific antibody for urothelial neoplasms. Because if KRT17 is seen in ≥10% of cells, its sensitivity is 89% and its specificity is 88% in distinguishing malignant lesions from normal urothelial mucosa (8).

In our study, there was a significant difference in the proportion of KRT17 positive cells between NBM and bladder neoplasms. We demonstrated that KRT17 positivity according to staining proportion alone is an important diagnostic factor in distinguishing neoplastic bladder lesions from NBM ($p<0.001$).

We identified that a cut-off of ≥2.5% of cells staining positive for KRT17, displayed a sensitivity of 86.3% and a specificity of 100% in discriminating neoplastic lesions from normal urothelial mucosa. If KRT17 was considered positive regardless of percentage and density, the sensitivity of KRT17 was 86.2 % and the specificity was 100 % in distinguishing neoplastic lesions from normal urothelial mucosa. We found that KRT17 was a reliable diagnostic marker in discriminating neoplastic bladder lesions from NBM.

Different expressions of SPINK1 in different carcinomas accompany poor prognosis (16-20).

Holah et al. suggested that SPINK1 expression was more pronounced in well-differentiated hepatocellular carcinoma (WD-HCC) than in high-grade dysplastic nodule (HGDN), and it could be used in the differential diagnosis of both lesions (21).

Our study showed that SPINK1 immunopositivity score of 2 at a minimum of 10% of tumor cells, was significantly higher in all neoplasms compared to normal urothelial mucosa. SPINK1 was expressed in highest proportion of cells in PUNLMP.

Hotakainen et al. (22) and Liu et al (23) also reported that SPINK1 expression was more prominent in low-grade bladder cancers and decreases with increasing tumor grade. Similarly, SPINK1 staining is more pronounced in low-grade tumors than high-grade tumors in colon cancers. In multivariate analysis, low SPINK1 expression and tumour differentiation are independent prognostic factors for adverse outcome (24). Rink et al. suggested that the loss of SPINK1 expression seen in urothelial carcinomas undergoing radical cystectomy was significantly associated with lymph node metastases and advanced tumor stages (25).

However, our study demonstrated that there was no statistically significant difference in the expression of SPINK1 between IUC-HG-NMI and IUC-HG-MI ($p=0.982$).

We found that a cut-off of ≥12.5% of cells staining positive for SPINK1, displayed a sensitivity of 62.6% and a specificity of 60% in discriminating neoplastic lesions from NBM. When SPINK1 was 2 positive at a minimum of 10% of tumor cells, the sensitivity of SPINK1 was 68.7 % and the specificity was 50 % in discriminating neoplastic lesions from NBM.

Our findings indicate that the mean proportion of KRT17 and SPINK1 positive tumor cells are associated with neoplastic development, but KRT17 and SPINK1 expression is reduced in infiltrating and high-grade urothelial tumors compared to non-invasive and low grade urothelial tumors.

In this study, 13 of 20 patients in the IUC-HG-MI group, who were in the 55 infiltrating urothelial carcinoma group, showed staining with laminin around the nests of the carcinoma cells, but not with CD31 and D2-40. This indicates that tumor cells sometimes mimic lymphovascular invasion by expressing laminin. In such suspicious patients, CD31 and D2-40 stains should be applied to accurately recognize lymphovascular invasion.

Laminins as a component of basement membrane are an essential class of proteins, as they affect cell differentiation, migration, adhesion, and even cell survival (26). Basement membrane is recognized as a cancer cell defense barrier, which inhibits the local infiltration of cancer cells by inhibiting the adhesion of cancer cells to it (27). Absence of laminin staining supports the presence of an invasive neoplasm.

Pradhan et al. demonstrated laminin staining in the basement membrane of 5 NBM and 25 noninvasive urothelial carcinomas in addition to perivascular areas in superficial and deep muscle tissue, but not in invasive and malignant urothelial cells (28). Many carcinomas, such as breast, lung, colorectal cancer, and squamous cell carcinoma, can express high levels of laminin (27).

We observed laminin staining in only 13 of 20 patients in the "infiltrating urothelial carcinoma, high-grade, muscle-invasive" group and all non-infiltrating tumors. Laminin staining was not observed in other infiltrating tumors. Laminin staining can mimic lymphovascular invasion. Therefore, both morphological findings and immunohistochemical panel consisting of Laminin, CD31 and D2-40 staining should be evaluated together to distinguish invasion from lymphovascular invasion.

We believe that Laminin staining should be performed to detect invasion, especially in non-invasive high-grade carcinomas, UCIS and in patients with suspected invasion.

Conclusion

KRT17 and SPINK1 are often expressed in bladder tumors. Both KRT17 and SPINK1 expression indicate a tumoral lesion whereas deficiency of both antibodies indicates a non-tumoral lesion. As a result, the positivity or negativity of both KRT17 and SPINK1 in tissue distinguishes neoplastic lesions from NBM with both high sensitivity and specificity. We think that the immunohistochemical panel consisting of KRT17, SPINK1 and Laminin will increase the rate of accurate diagnosis in bladder tumors.

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