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CD10 expression in urothelial bladder carcinomas: staining patterns and relationship with pathologic parameters*

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Aim: CD10 is a cell surface metalloprotease that inactivates various bioactive neuropeptides. CD10 has been suggested as a useful prognostic marker for urothelial carcinoma, but there are only a few studies of CD10 in urothelial tumors and they have shown varying results. The aims of this study were to investigate the expression of CD10 in urothelial bladder carcinomas and to clarify its association with pathologic parameters.

Materials and methods: A total of 50 urothelial bladder carcinomas were selected from archival material. All cases were reevaluated histopathologically and graded according to the WHO/ISUP 1998 classification. The TNM system was used for their pathological staging. CD 10 immunohistochemical staining was performed on one representative tissue block for each case.

Results: 35 of the 50 (70%) tumors showed positive CD10 immunostaining. We found a statistically significant difference in terms of CD10 extent between low grade and high grade tumors. No association was detected between CD10 expression and other pathologic parameters. No relationship was found between CD10 expression and tumor size, lymphovascular invasion, or pathological stage.

Conclusion: According to our findings, high grade carcinomas showed a wider CD10 expression than that in low grade carcinomas. CD10 may play an important role in the progression and differentiation of bladder urothelial carcinomas.

Key words: Bladder, papillary urothelial carcinoma, CD10, immunohistochemistry

Ürotelyal mesane karsinomlarında CD10 Ekspresyonu: Boyanma paterni ve patolojik parametreler ile ilişkisi

Amaç: CD10, çeşitli biyoaktif nöropeptitleri inaktive eden hücre yüzey metaloproteazıdır. CD10'un ürotelyal karsinomlarda prognostik belirleyici olarak kullanılabileceği öne sürülmektedir. Ancak ürotelyal tümörlerde CD10 ekspresyonunu araştıran az sayıda çalışma bulunmaktadır ve sonuçları farklılıklar göstermektedir. Bu çalışmada, ürotelyal mesane karsinomlarında CD10 ekspresyonu incelenmiş ve patolojik parametreler ile ilişkisi araştırılmıştır.

Yöntem ve gereç: Toplam 50 ürotelyal mesane karsinomu arşiv materyallerinden seçilmiştir. Tüm olgularda histopatolojik bulgular tekrar gözden geçirilmiş ve WHO/ISUP 1998 sistemine göre derecelendirme uygulanmıştır. Patolojik evreleme için TNM sistemi kullanılmıştır. Her olgu için seçilen bir temsili doku bloğuna CD10 için immünhistokimyasal boyama işlemi gerçekleştirilmiştir.

Bulgular: Elli olgununun 35 (% 70)'inde CD10 ekspresyonu belirlendi. Çalışmamızda düşük ve yüksek dereceli ürotelyal tümörler arasında CD10 immünreaktivitesinin yaygınlığı açısından istatistiksel olarak anlamlı farklılık belirlendi. CD10 ekspresyonu ile tümör boyutu, lenfovasküler invazyon varlığı ve patolojik evre arasında ilişki saptanmadı.

Sonuç: Bulgularımız, yüksek dereceli ürotelyal karsinomlarda, düşük dereceli karsinomlara göre daha yüksek oranda CD10 ekspresyonu saptandığını göstermektedir. CD10 ürotelyal karsinomların progresyonunda ve diferansiyasyonunda rol oynayabilir.

Anahtar sözcükler: Mesane, papiller ürotelyal karsinom, CD10, immünhistokimya

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Introduction

CD10 is a cell-surface metalloprotease that can limit cellular responses to peptide hormones by hydrolyzing them, reducing the concentration of peptide available for receptor binding and signal transduction. In addition to its enzymatic function, the CD10 protein has a direct role in signal transduction pathways that regulate cell growth and apoptosis. Because of its structural similarity to the matrix metalloproteases in the stroma, CD10 is thought also to affect the invasion and metastatic potential of tumor cells by altering the cellular microenvironment (1-3). CD10 was initially identified as a cell-surface antigen expressed by acute lymphoblastic leukaemias, and hence its early designation as CALLA. This antigen is widely used for the categorization of acute leukaemias and for the subclassification of malignant lymphomas (3-5). Recently, several studies have reported CD10 expression in various non-hematolymphoid tumors and in normal tissues (5-9).

Changes in CD10 activity have been demonstrated to have different effects in different tumor types (5-9). CD10 expression inhibits the proliferation and progression of tumor cells in prostate (10-11) and lung cancers (12), stimulating apoptosis. In contrast, increases in CD10 activity in colorectal carcinomas (13), breast carcinomas (14), and malignant melanomas (15) are associated with invasion and metastasis.

To date, few studies have investigated CD10 expression in urinary bladder tissues. Whilst some studies have not detected CD10 expression in nonneoplastic urothelium (9), others have, albeit to a lesser extent than in urothelial tumors (16-17). Its expression has been reported to occur in 43%–67% of urothelial neoplasms. Whilst in the majority of reports, CD10 expression shows an inverse correlation with tumor grade, a positive correlation with grade has been noted in others (9, 16-18).

In this study, we investigated CD10 expression in urothelial carcinomas by immunohistochemistry and analyzed its relationship with pathological parameters, such as tumor size, histologic grade, pathologic stage, and lymphovascular invasion.

Materials and methods

Patients

We randomly selected 50 patients with urinary bladder urothelial carcinoma from pathology archives. Of these, 46 were transurethral resections (TUR) and 4 were radical cystectomies performed at Zonguldak Karaelmas University, Faculty of Medicine and Lutfi Kırdar Kartal Training and Research Hospital, in 2005 and 2006. In addition, material from 10 cystoscopic biopsies, which was obtained in non-neoplastic disease and contained normal urothelial mucosa, was included in the study for control purposes. We obtained the patients' clinical data from hospital charts. In determining the tumor size, the cystoscopy reports and macroscopic sizes of the tumors present in the pathological specimens were taken as the basis for cystectomy and TUR material, respectively.

Pathology

Haematoxylin and eosin-stained slides from each case were reevaluated histopathologically. All cases were reviewed for tumor size, histologic grade, pathologic stage, and lymphovascular invasion. For tumor grading, according to the World Health Organization/International Society for Urological Pathologists (WHO/ISUP) - 1998 classification, urothelial carcinomas were subdivided into low-grade papillary urothelial carcinoma (LGPUC) and high-grade papillary urothelial carcinoma (HGPUC). The TNM system was used for pathologic staging: Ta, noninvasive papillary urothelial carcinoma; T1, tumor has invaded subepithelial connective tissue; T2, tumor has invaded the muscularis propria; and T3, the tumor has invaded the perivesical tissue.

Immunohistochemistry

Expressions of CD10 were tested on formalin-fixed, paraffin-embedded section from 50 urinary bladder urothelial carcinoma tissues and 10 nonneoplastic urothelial mucosae. For the immunohistochemical studies, immunostaining was performed using the streptavidin-biotin-peroxidase complex technique. Sections were placed on positively charged glass slides, deparaffinized in xylene, and

hydrated in graded alcohol series. They were then left to boil in 10 mm citrate buffer saline (pH 6.0) for 20 min and washed with phosphate-buffered saline (PBS, pH 7.3). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. The primary antibody used was monoclonal mouse CD10 antibody clone 56C6 (Neomarkers, Fremont, CA, USA) at a 1/60 dilution incubated 4 h at room temperature. After washing in phosphate-buffered saline with Tween-20, the tissues were incubated with a biotin-conjugated secondary antibody and then incubated using the streptavidin-biotin system for 30 min at room temperature. The reactions became visible after immersion of the specimens in 3,3-diaminobenzidine tetrahydrochloride (DAB). The sections were counterstained with Mayer's hematoxylin stain, and then rinsed and mounted. Appropriate positive (tonsil tissue) and negative controls (primary antibody omitted) were included simultaneously in the stainings.

Evaluation of immunostaining

Immunohistochemical staining was evaluated in a semiquantitative fashion independently by 2 pathologists (N.O.K. and B.B.), who were blinded to the clinicopathological data. Discrepancies were resolved by simultaneous re-examination of the slides by both investigators using a double-headed microscope. Membranous and cytoplasmic membrane staining was considered positive with a 1% cut-off point in tumor cells. The extent of immunoreactivity was scored semiquantitatively according to the following criteria: Negative, 1% or fewer positive cells; 1+, 2%–10% positive cells; 2+, 11%–50% positive cells; 3+, more than 50% positive cells.

Statistical analysis

The relationship between CD10 positivity score and tumor size, histologic grade, pathologic stage, and lymphovascular invasion were evaluated statistically. The data were compiled and analyzed using SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance of the results was evaluated using chi-square tests. P values <0.05 were considered significant.

Results

Clinicopathologic characteristics (Table 1)

The age of the patients ranged from 23 to 89 years (mean ± SD: 67.2 ± 12.1). The subjects consisted of 46 (92%) men and 4 (8%) women. Based on pathologic staging, 18 (36%), 17 (34%), and 15 (30%) patients were ranked at stages pTa, pT1, and pT2, respectively. No patients were at stage pT3. Twenty three (46%) patients were low-grade and 27 (54%) were high-grade. Tumor size ranged from 0.5 to 7 cm (mean ± SD: 4.21 ± 1.54). Lymphovascular invasion was detected in 18 (36%) cases.

Table 1. Clinic, pathologic, and immunohistochemical characteristics.

Parameters	No (%) (n = 50)	CD10 (+) (n = 35)
Sex		
Male	46 (92%)	34 (97.1%)
Female	4 (8%)	1 (2.9%)
Age		
Range	23-89	
Mean ± SD	67.2 ± 12.1	
Tumor size		
Range	0.5-7cm	
Mean ± SD	4.21±1.54	
Histological grade		
LGPUC	23 (46%)	9 (25.7%)
HGPUC	27 (54%)	26 (74.3%)
Pathological stage		
pTa	18 (36%)	12 (34.3%)
pT1	17 (34%)	10(28.6%)
pT2	15 (30%)	13 (37.1%)
pT3	0 (0%)	0(%0)
Lymphovascular invasion		
Positive	18 (36%)	11 (31.4%)
Negative	32 (64%)	24 (68.6%)

LGPUC: Low- grade papillary urothelial carcinoma,
HGPUC: High- grade papillary urothelial carcinoma.
Ta: Noninvasive papillary urothelial carcinoma,
T1: Tumor has invaded subepithelial connective tissue,
T2: Tumor has invaded the muscularis propria,
T3: Tumor has invaded the perivesical tissue.

Immunohistochemical findings

Thirty-five of the 50 (70%) patients demonstrated positive CD10 immunostaining: +1, +2, and +3 reactivity were observed in 8 (16%), 21 (42%), and 6 (12%) tumors, respectively. The remaining 15 patients (30%) were negative for CD10. No staining was seen in nonneoplastic urothelium (Figure 1). Staining pattern in positive carcinomas was diffuse cytoplasmic in 22 patients (62.9%) and cytoplasmic and membranous in 13 patients (37.1%) (Figure 2). None of the cases exhibited an apical/luminal staining pattern. HGPUCs exhibited more extensive CD10 expression than LGPUCs and this was statistically significant (P = 0.0001) (Table 2). In 39.1% (9/23) of the LGPUCs, positive CD10 immunoreactivity was detected: the immunoreactivity was 1+, 2+, and 3+ in 66.7% (6/9), 33.1% (3/9), and 0% (0/9), respectively. By contrast, a positive CD10 reaction was observed in 96.3% (26/27) of the HGPUCs, and the immunoreactivity was 1+, 2+, and 3+ in 7.8% (2/26), 69.2% (18/26), and 23% (6/26) of these patients, respectively. On the other hand, no significant association between pathologic stage and CD10 score was found (P = 0.10). CD10 expression did not differ in terms of tumor size or presence of lymphovascular invasion.

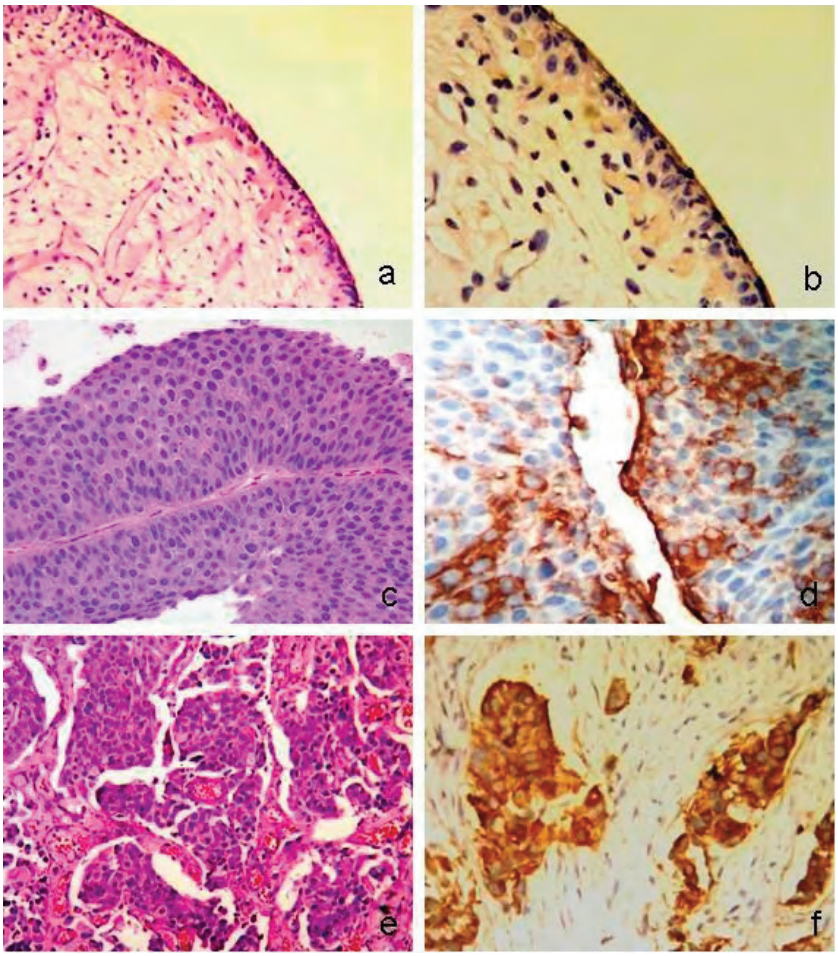

Figure 1. a) Nonneoplastic urothelium (Hematoxylin & Eosin [H&E], ×100), b) Negative CD10 immunoreactivity in the nonneoplastic mucosa (Score 0; Strept–Avidin–Biotin–Peroxidase Complex [SABC], Diaminobenzidine [DAB], ×200), c) LGPUC (H&E, ×200), d) Focal cytoplasmic CD10 immunoreactivity in LGPUC (Score 2+; SABC, DAB, ×400), e) HGPUC (H&E, ×100), f) Diffuse cytoplasmic and focal membranous immunoreactivity of CD10 in HGPUC (Score 3+; SABC, DAB, ×400).

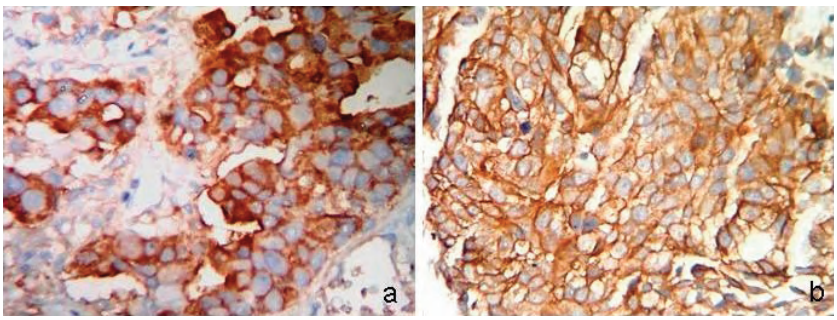

Figure 2. Immunoreactivity of CD10 in HGPUC: a) Diffuse cytoplasmic staining (Score 3+, SABC, DAB, ×1000); b) Cytoplasmic and membranous staining (Score 3+; SABC, DAB, ×1000).

Table 2. Extent of CD10 immunoreactivity with respect to histological grade and pathological stage of tumors (n = 50).

Parameters	Extent of CD10 immunoreactivity score				Total	Test of. Sig.
	0	1	2	3		
Histological grade						
LGPUC	14 (60.9%)	6 (26.1%)	3 (13%)	0 (0%)	23 (46%)	
HGPUC	1 (3.7%)	2 (7.4%)	18 (66.7%)	6 (22.2%)	27 (54%)	
Total	15 (30%)	8 (16%)	21 (42%)	6 (12%)	50 (100%)	P = 0.0001*
Pathological stage						
pTa	6 (33.3%)	5 (27.8%)	7 (38.9%)	0 (0%)	18 (36%)	
pT1	7 (41.2%)	2 (11.8%)	6 (35.2%)	2 (11.8%)	17 (34%)	
pT2	2 (13.3%)	1 6.7%)	8 (53.3%)	4 (26.7%)	15 (30%)	
pT3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Total	15 (30%)	8 (16%)	21 (42%)	6 (12%)	50 (100%)	P = 0.10

LGPUC: Low- grade papillary urothelial carcinoma,
HGPUC: High- grade papillary urothelial carcinoma.
Ta: Noninvasive papillary urothelial carcinoma, T1: Tumour has invaded subepithelial connective tissue, T2: Tumour has invaded the muscularis propria, T3: the tumour has invaded the perivesical tissue.
* P < .0.5 for LGPUCvs HGPUC.

Discussion

The CD10 molecule plays a role in the regulation of cell growth, differentiation, adhesion, invasion, and angiogenesis via enzymatic hydrolysis of various peptide hormones (1-3). All of these processes are important in normal physiology, as well as in tumor growth and progression. Studies conducted on human tumors reveal that CD10 activity exerts different influences on different tumor types (5-9). Loss of CD10 expression in prostate (10-11), lung (12), and endometrial tumors (19) accelerates tumor development and progression, and in these tumors, CD10 is thought to function as a tumor suppressor. In melanomas (15), breast cancers (14), and colon (13) tumors, CD10 expression is associated with progression and metastasis.

Few studies have investigated CD10 activity in urinary bladder tissues, and their results have differed

markedly. Koiso et al. detected the presence of CD10 enzyme activity and immunoreactivity in a urinary bladder cancer cell series (18). In this study, greater activity and expression was reported in superficial carcinomas compared to invasive carcinomas. McIntosh et al. developed a CD10 monoclonal antibody appropriate for use in formalin-fixed and paraffin-embedded human tissues (9). These researchers investigated various normal tissues and reported the absence of CD10 immunoreactivity in nonneoplastic urothelium. In the same study, CD10 expression was detected in 54% of urothelial carcinomas, but the relationship between expression and pathologic stage or histologic grade was not investigated. Chu et al. reported that CD10 is more widely expressed in noninvasive urothelial carcinomas than in invasive carcinomas (6). Murali et al. detected CD10 expression in 50% of nonneoplastic urinary bladder mucosa samples and in 67% of urothelial neoplasms. They characterized CD10 expression as a parameter associated with high histologic grade, but independent of tumor stage (17). Bircan et al. detected CD10 expression in 9.9% of nonneoplastic urothelium and in 43% of urothelial tumors (16). In that study, while no relationship was found between CD10 expression and histologic grade, an inverse correlation was reported between CD10 expression and tumor stage.

Three different staining patterns have been detected in various tumor types expressing CD10: an apical/luminal CD10 staining pattern, a diffuse cytoplasmic pattern, and a membranous/Golgi pattern (6-7). In urothelial tumors, cytoplasmic, and membranous patterns are more frequently observed (6, 16-17).

Amongst studies on urothelial tissues, different CD10 reaction patterns have been tracked, and different limit values accepted in the scoring of CD10-positivity. In some studies, only membranous staining was recognized as significant (20), whilst most assessed cytoplasmic, membranous and/or apical staining patterns (9, 16-18). Whilst certain studies classed all samples in which more than 1% of tumor cells were stained as positive (20), most studies required at least 10% positive cells in a sample (16-17). These differences affect the positivity rate of CD10 expression in urinary bladder tumors. In our study, samples in which more than 1% of tumor cells showed cytoplasmic and/or membranous staining were accepted as positive.

Previous studies on urothelial tumors have reported membranous and cytoplasmic staining patterns mostly in neoplastic or dysplastic urothelial epithelium, however apical/luminal staining patterns is seen more frequently in nonneoplastic urothelium (17-18). Some investigators suggest that cytoplasmic CD10 immunoreactivity may be an indicator of the neoplastic alterations in urothelial epithelium. We detected diffuse cytoplasmic reactions in 62.9% of CD10 positive urothelial carcinomas and cytoplasmic /membranous reactions in the remaining positive cases. None of our cases showed purely membranous or apical/luminal staining patterns. Our findings, taken together with the results of previous studies, suggest that cytoplasmic or cytoplasmic/membranous CD10 immunoreactivity may be an indicator of neoplastic changes in urothelial lesions.

Numerous genetic changes have been described in urothelial tumors. Genetic aberrations on the long arm of chromosome 3, where the CD10 gene is located, are detected in 7–24% of invasive urothelial carcinomas and in 1–5% of noninvasive urothelial tumors (20-21). More genetic changes are known to occur in high-grade tumors than in low-grade tumors. In urothelial neoplasms CD10 expression is reported at rates ranging from 43% to 67%. In this study, we detected CD10 expression in 70% of urothelial carcinomas. Similar to the findings of Murali et al. (11), we detected more prevalent CD10 immunoreactivity in cases with high histologic grade. This could be a consequence of genetic changes causing wild-type or mutant CD10 expression in the high-grade carcinomas. Our findings suggest that CD10 expression is a parameter associated with halted differentiation in urothelial tumors.

Various growth factors that are influential in the pathogenesis and progression of urothelial tumors have been described. The beginning of angiogenesis is an important step in the transition from a superficial papillary phenotype to that of an invasive tumor. CD10 is known to hydrolyze endothelin, and may play a role in urothelial tumor angiogenesis and invasion by regulating endothelin levels (20-24). Most studies have reported an inverse correlation between CD10

expression and pathologic stage. Murali et al. (17), however, have advocated that CD10 expression is a parameter independent of stage. In the present study, whilst CD10 expression was observed more often in invasive tumors, a statistically significant difference was not detected. We believe that the low number of cases in our study may have contributed to this result. Comprehensive studies including more cases may be needed to clarify the effect of CD10 in the invasion process of urothelial carcinomas.

Our findings show that urothelial carcinomas express CD10, and that this is associated more frequently with poorly differentiated tumor histology. Future studies conducted in larger patient groups and supported by additional molecular techniques should provide the information needed to clarify the role of CD10 expression in the pathogenesis and progression of urothelial tumors.

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