Turkish Journal of Pathology 2008;24(3):147-152

CD44 and MMP-2 expression in urothelial carcinoma

Ürotelyal karsinomda CD44 ve MMP-2 ekspresyonu

Gülgün ERDOĞAN[1], İlknur KÜÇÜKOSMANOĞLU[1], Bahar AKKAYA[1], Türker KÖKSAL[2], Gülten KARPUZOĞLU[1]

Akdeniz University, School of Medicine Department of Pathology[1] and Urology[2], ANTALYA

ABSTRACT

Aim: CD44, one of the adhesion molecules, is thought to play an important role in cell-cell and cell-matrix interactions. Matrix metalloproteinases are degradative enzymes that remodel extracellular components. In this study the relation of MMP-2 and CD44 expressions with the histologic classification and the pathologic stage of urothelial carcinoma was revealed using immunohistochemistry.

Material and Methods: Thirty-nine patients with urothelial carcinoma of the bladder were studied. The histological classification was performed according to WHO criteria. Patients were grouped as infiltrating urothelial carcinoma, low grade non-invasive papillary urothelial carcinoma, and high grade non-invasive papillary urothelial carcinoma. The pathological staging was done according to the TNM classification. Immunohistochemical staining using CD44 and MMP-2 antibodies was performed on tissue blocks.

Results: CD44 immunoreactivity was detected in 77% (30/39) of the tumours which was significantly higher in non-invasive papillary urothelial carcinomas, low grade non-invasive papillary urothelial carcinomas, high grade infiltrating urothelial carcinomas ($p \geq 0.05$). MMP-2 expression was observed in 69% (27 of 39) of the tumours. There were no significant differences in MMP-2 expression between various histologic subtypes and non-invasive and infiltrative tumours.

Conclusion: In conclusion, higher expression of CD44 is inversely correlated with infiltrative potential of urothelial carcinoma. These results should be supported by further studies.

Key words: CD44, MMP-2, urothelial carcinoma

ÖZET

Amaç: CD44 hücreler arası ve hücre matriks ilişkisinde önemli rol oynayan bir adezyon molekülüdür. Matriks metalloproteinazlar ise ekstrasellüler matriks komponentlerinin yıkımından sorumlu bir enzimdir. Bu çalışmada ürotelyal karsinomlarda immünhistokimyasal olarak MMP-2 ve CD44 ekspresyonu ve bu verilerin histolojik alt tip ve patolojik evre ile olan ilişkisinin araştırılması planlandı.

Materyal ve Metod: Ürotelyal karsinom tanısı almış 39 olguya ait parafin bloklar çalışıldı. Tümör histolojik alt tipi WHO kriterlerine göre belirlendi. Olgular infiltratif ürotelyal karsinom, düşük dereceli non-invaziv papiller ürotelyal karsinom, yüksek dereceli non-invaziv papiller ürotelyal karsinom, olmak üzere gruplara ayrıldı. Tümör kesitlerine immünhistokimyasal olarak CD44 ve MMP-2 primer antikorları uygulandı.

Bulgular: CD44 ile 77% (30/39) oranda pozitif boyanma izlendi. Ayrıca düşük dereceli non invasiv ürotelyal karsinomlarda, yüksek dereceli non-invaziv papiller ürotelyal karsinom ve infiltratif ürotelyal karsinomlara göre istatistiksel olarak anlamlı yüksek bulundu ($p \geq 0.05$). MMP-2 ekspresyonu ise 69% (27/39) oranda görüldü. MMP-2 ekspresyonu ile histolojik alt gurup ve yüzeyel ve invaziv tümör özellikleri arasında anlamlı ilişki izlenmedi.

Sonuç: Sonuç olarak ürotelyal karsinomlarda CD44 ekspresyonu tümörün infiltratif özelliği ile ters orantılıdır. Bu sonuç daha geniş serilerde yapılan çalışmalar ile desteklenmelidir.

Anahtar sözcükler: CD44, MMP-2, ürotelyal karsinom

Received: 10.04.2008
Accepted: 06.06.2008
Corresponding Author: Gülgün Erdoğan, Akdeniz University, School of Medicine Department of Pathology, 07070, Antalya

INTRODUCTION

Urothelial carcinoma of the urinary blad-

der is the 2nd most frequent malignancy of the genitourinary tract (1). Superficial, low grade tumors have higher recurrence rates and higher grade tumors have higher invasive potential and tendency to metastatic potential (2,3,4) Generally, standard histopathological characteristics of urothelial cancers, including tumor grade and stage determine the tumor's behaviour but description of new tumor characteristics may be helpful for the patients' treatment.

Preservation of intercellular adhesion is critical to the maintenance of normal tissue architecture. Distruption of intercellular adhesion in tumour cells is considered as the first step in the process of invasion of the surrounding tissues and metastasis to distant organs. CD44, one of the adhesion molecules which is a transmembrane glycoprotein, is thought to play an important role in cell-cell and cell-matrix interactions (5,6). Loss of expression of these adhesion molecules may contribute to progression and metastasis formation in various human malignancies such as squamous cell carcinoma of the skin, breast and lung carcinoma (5,6). In the literature there are some studies about CD44 expression in bladder carcinoma. Their data show that an inverse correlation between the expression of CD44 and histological grade and tumour stage exists and loss of CD44 variants may provide an additional parameter in identifying patients with urothelial carcinoma at risk for tumour recurrence (6,7,8). In addition Kuncova et al reported that CD44 v6 expression correlated with a higher proliferative activity and cellular atypia (9).

Degradation of the extracellular matrix and basement membrane is essential for tissue invasion by tumor cells and metastasis at the various sites, and also it depends on numerous extracellular proteinases, whose properties are controlled by proenzymes and specific inhibitors (10). During this process, tumour cells secrete increased amounts of degradative enzymes (11). Matrix metalloproteinases are degradative enzymes that remodel extracellular components in healthy and diseased tissue (11,12). Under normal physiological conditions they play an important role in embryonic growth and development and they are involved in all steps during physiologic repair and dynamic reorganisation (13). Elevated levels of MMPs have been found in pathologic situations such as rheumatoid arthritis, arteriosclerosis, hepatitis and several types of human cancers (13). In the literature numerous studies have shown an association between tumor growth and metastasis and MMP expression (11-13). Their expressions are elevated in many types of human cancers, such as breast, lung, gastric, ovarian, brain and bladder cancer (10). MMPs are well known proteases that degrade particularly the basement membrane collagene and their effects are controlled by proenzymes and specific inhibitors. MMPs are a family of zinc dependent proteases and MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) are well known types for their ability to cleave the helical domains of type IV collagen (13). In recent studies, the expression of MMP-2 and MMP-9 have been observed in high stage and grade tumours and highly correlated with the agressiveness of bladder cancer (10-13).

In this study the relation of the MMPs and CD44 expression with the histologic classification and the pathologic stage of urinary bladder urothelial carcinoma was revealed using immunohistochemical methods.

MATERIALS AND METHODS

Thirty-nine patients with urothelial carcinoma of the bladder were retrieved from the archives of Department of Pathology of Akdeniz University from 1999 to 2002. The mean age of our patients was 61 years (range 46-74). The histological classification was performed according to WHO criteria. Patients were grouped as infiltrating urothelial carcinoma, non-invasive low grade papillary urothelial carcinoma, non-invasive high grade papillary urothelial carcinoma. The pathological staging was done according to the TNM classifications. The tumours

were grouped as non-invasive papillary urothelial carcinoma (Ta) and invasive tumours (T1, T2, T3 and T4). Among 39 cases,19 cases were classified as non-invasive low grade papillary urothelial carcinoma, and 9 non-invasive high grade papillary urothelial carcinoma, and 11 infiltrating urothelial carcinoma, while 28 were classified as Ta and 11 as invasive tumours.

Formalin-fixed, paraffin embedded tissue sections representative of the tumour in each case were immunostained with CD44 (clone DF1485 code7085, Dako, Denmark) and MMP-2 (clone Vıd2, code MS 136 P, Neomarker, USA). Tissue sections were deparaffinized, rehydrated, and microwave antigen retrieval in EDTA buffer (AF9004500, Labvision, CA) was performed. For 30 minutes pH 8.0 was maintained. The sections were then incubated with polyclonal anti-CD44 and MMP-2 at a dilution of 1: 200 for 60 minutes. The Ultravision detection system and anti-mouse, HRP/DAB were used. Sections were developed with diaminobenzidine tetrahydrochloride substrate, and counterstained with hematoxylin. The immunostained slides were evaluated by semiquantative method and cytoplasmic membranous immunostaining for CD44 and cytoplasmic staining for MMP-2 were evaluated. According to the extent and the intensity of staining for CD44 positive cases, they were divided into three categories: focally positive or slightly heterogenous staining was scored 1; 2, for strong heterogenous or 3, for strong homogenous staining. The extent of staining were scored 1-3 in MMP-2 positive tumours. The extent of staining were scored as the percentage of the tumoral area stained as 1 (≤ 25%), 2 (25-75%), and > 75%), respectively.

T test and Mann-Whitney U tests were used for statistical analysis, evaluating the relationship between positive staining and tumour grade and stage.

RESULTS

CD44 immunoreactivity was detected in 77% (30 out of 39) of the tumours. CD44 expression is summarized in Table 1. All of the non-invasive low grade papillary urothelial carcinomas, and 55% (5/9) of the non-invasive high grade papillary urothelial carcinomas, 85% (24/28) of the non-invasive and 55% (6/11) of the invasive ones expressed CD44 (Figure 1,2). CD44 expression was significantly higher in non-invasive papillary urothelial carcinomas, low grade non-invasive papillary urothelial carcinomas, and higher grade infiltrating urothelial carcinomas ($p \geq 0.05$). We observed that higher expression of CD44 was inversely correlated with infiltrative potential of the tumour.

Table 1. CD44 and MMP-2 immunopositivity in urothelial carcinoma.

	CD44 % (n)	MMP-2 % (n)
Non-invasive papillary urothelial carcinoma, Low grade	100% (19/19)	79% (15/19)
Non-invasive papillary urothelial carcinoma, High grade	55% (5/9)	55% (5/9)
Superficial	89% (24/28)	71% (20/28)
Invasive	45% (6/11)	63% (7/11)

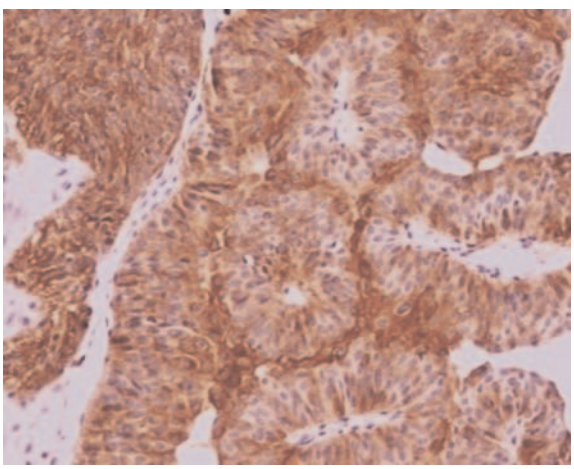

Figure 1. CD44 immunopositivity in non-invasive papillary urothelial carcinoma, low grade (DAB, x100).

MMP-2 expression was observed in 69% (27 of 39) of the tumours. MMP-2 positivity was found in 79% (15/19) of the low grade non-

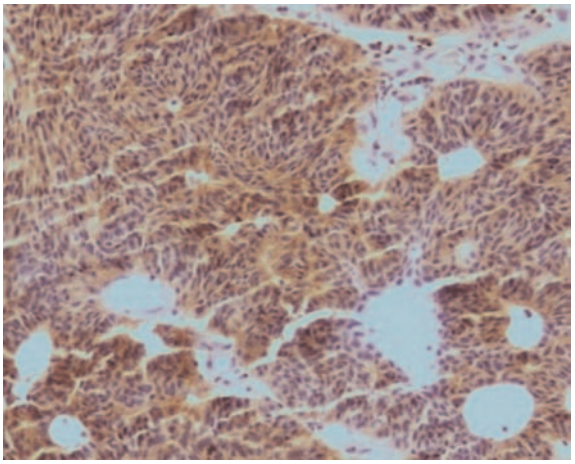

Figure 2. CD44 heterogenity in non-invasive papillary urothelial carcinoma, high grade (DAB, X100).

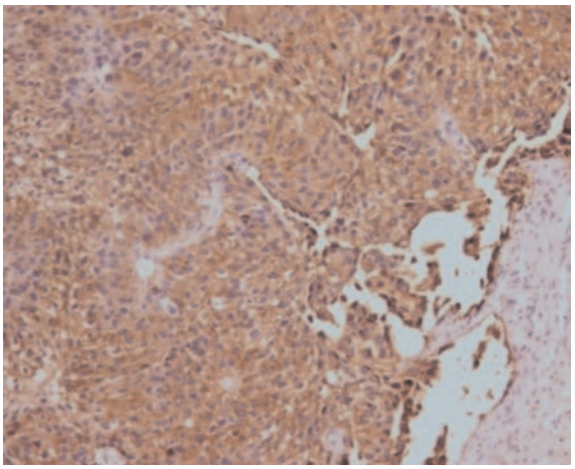

Figure 3. MMP-2 immunopositivity in infiltrative urothelial carcinoma (DAB, X100).

invasive papillary urothelial carcinomas, and 55% (5/9) of the non-invasive high grade papillary urothelial carcinomas, and 63% (7/11) of the invasive ones (Figure 3). Expression of MMP-2 was detected in 71% (20/28) of non-invasive and 63% (7/11) of invasive tumours There were no significant differences in MMP-2 expression between various histologic subtypes and non-invasive and infiltrative tumours.

However, expression rate of MMP-2 was relatively higher in low grade tumours.

DISCUSSION

Transitional cell carcinoma (TCC) of the bladder is a heterogenous disease and many of them are superficial tumors and possibly less aggressive biological behaviour than invasive ones and many of which may be as aggressive as their invasive counterparts. The mechanism of recurrences, tumor invasion and metastasis is associated with complex biological processes that remain unclear. Staging and grading allow a certain degree of stratification of biological potential of the tumour and also useful for clinical purposes. However, tumoral heterogenity still exists within various prognostic subgroups.

Distruption of intercellular adhesion in tumour cells can result in dissociation, invasion and metastasis. CD44 is a widely expressed cell surface adhesion molecule in which various isoforms are related during cancer initiation and progression (6,7). Previously published data on CD44 expression in TCC showed inverse correlation between tumor stage and CD44. Immunohistochemistry gave an additional tool for identfying patients at high risk for recurrence of prostatic TCC (6). In addition, CD44 expression was reduced in poorly differentiated and invasive transitional cell carcinoma as compared to well or moderately differentiated and noninvasive tumours (7). In another study there was no correlation between CD44 variant expression and clinicopathologic criteria such as stage, grade and survival (5). Stravropoulos et al reported that the expression intensity CD44(s) was statistically significantly higher in tumors of intermediate grade than grade 3 disease (14). Although higher expression was not detected in Ta tumors compared to T1, there was significantly more extensive positive areas in noninvasive tumors than the deeply invasive tumors (14). In addition, loss of expression of CD44 standard form in higher grade and advanced stage provided evidence of the involvement of CD44 in tumorogenesis of TCC and an unfavorable outcome in terms of progression in superficial bladder cancer (14).

In another reported study, CD44 v6 expression correlated with a higher proliferative

activity and cellular atypia (9). Kuncova et al said that, progression to higher grades of TCC was associated with a decrease in CD44 expression, higher proliferative activity of tumour cells, and more frequent p53 over expression (15). Our current findings on CD44 expression are in agreement with previously reported studies that higher CD44 expression was revealed in low grade and noninvasive tumors. In addition, these findings support evidence indicating involvement of CD44 in the tumorogenesis of TCC.

Matrix metalloproteinase plays an important role in degradation of extracellular matrix, which is an essential step in the cascade of metastasis. In the literature, there are a lot of studies which investigated associations among MMPs, tissue inhibitors and activators in several malignancies (10,11,12). In addition overexpression of MMP-2 has been found to be associated with higher stage and increased metastatic potential in a variety of tumors (11). Nakanishi et al reported positive relationship between the expression of MMP-2 and stage but showed limited value in predicting the progression of TCC (11).

In other studies referred, zymographical analysis of level of MMP-2 and MMP-9 showed a statistically significant increase in high grade and invasive tumors than in low grade and superficial tumors (13,16,17).

Another study indicated that MMP-2 and MMP-9 were strongly expressed in the tumors of patients with recurrent superficial TCCs of the bladder. Authors said that these markers may be used as a predictors of recurrences in superficial TCC (12).

Kanayama et al observed that high expression of MMP-2 or tissue inhibitor of metalloproteinase-2 significantly correlated with pathologic stage and higher level of MMPs and TIMPs expression was associated with decreased survival. Moreover the authors mentioned that MMPs are useful prognostic parameters in TCC and might be helpful in creating treatment protocols (18). Kexin et al reported that MMP-2/TIMP ratio might play a significant role in prediction of the agresiveness and prognosis of bladder tumors (19).

Our study shows that there is no correlation between MMP-2 expression and pathologic stage and grade. Differences between the results of our study and also previously reported studies may be due to the restricted number of cases and the heterogenity of the cases in the groups.

Another matrix metalloproteinase other than MMP-2 may play a major role in the processes of invasion and metastasis, and also MMPs may have an unknown complex role in that cascade and these hypothesis should be supported by sophisticated studies.

In conclusion, we observed that higher expression of CD44 is inversely correlated with infiltrative potential of urothelial carcinoma. We think that these results should be supported by further studies.

REFERENCES

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer Statistics, 2005. CA Cancer J Clin 2005;55:10-30.
2. Williams SG, Stein JP. Molecular pathways in bladder cancer. Urol Res 2004;32:373-385.
3. Pow-Sang JM, Seigne JD. Contemporary management of superficial bladder cancer. Cancer Control 2000;7:335-339.
4. Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol 2001;9:666-675.
5. Garcia del Muro X, Torregrosa A, Muñoz J, Castellsagué X, Condom E, Vigués F, et al. Prognostic value of the expression of E-cadherin and B-cathenin in bladder carcinoma. Eur J Cancer 2000;36;357-362.
6. Toma V, Hauri D, Schmid U, Ackermann D, Maurer R, Alund G, et al. Focal loss of CD44 variant protein expression is related to recurrence in superficial bladder carcinoma. Am J Pathol 1999;155:1427-1432.
7. Ross JS, del Rosario AD, Bui HX, Kallakury BV, Okby NT, Figge J. Expression of the CD44 cell adhesion in urinary bladder transitional cell carcinoma. Mod Pathol 1999; 9: 854-860.
8. Chuang CK, Liao SK. Differantial expression of CD44 variant isoforms by cell lines and tissue specimens of transitional cell carcinomas. Anticancer Res 2003; 23: 4635-4639.
9. Kuncová J, Kostrouch Z, Viale M, Revoltella R, Mandys V. Expression of CD44v6 correlates with cell proliferation and cellular atypia in urothelial carcinoma

cell lines 5637 and HT1197. Folio Biol 2005;5:3-11.
10. Gerhards S, Jung K, Koenig F, Daniltchenko D, Hauptmann S, Schnorr D, Loening SA. Expression of matrix metalloproteinase 2 and 9 in urine is associated with a high stage and grade of bladder carcinoma. Urology 2001;57:675-679
11. Nakanishi K, Kawai T, Sato H, Aida S, Kasamatsu H, Aurues T, Ikeda T. Expression of matrix metalloproteinase-2 and membrane-type-1-matrix metalloproteinase in transitional cell carcinoma of the upper urinary tract. Hum Pathol 2000;31:193-200.
12. Hara I, Miyake H, Hara S, Arakawa S, Kamidono S. Significance of matrix metalloproteinases and tissue inhibitors of metalloproteinase expression in the recurrence of superficial transisyonel cell carcinoma of the bladder. J Urol 2001;165:1769-1772.
13. Papathoma AS, Petraki C, Grigorakis A, Papakonstantinou H, Karavana V, Stefanakis S, et al. Prognostic significance of matrix metalloproteinase 2 and 9 in bladder cancer. Anticancer Res 2000; 20:2009-2014.
14. Stavropoulos NE, Filliadis I, Ioachim E, Michael M, Mermiga E, Hastazeris K, Nseyo UO. CD44 standard form expression as a predictor of progression in high risk superficial bladder carcinoma. Int Urol Nephrol 2001;33:479-483.
15. Kuncová J, Urban M, Mandys V. Expression of CD44s and CD44v6 in transitional cell carcinomas of the urinary bladder: comparison with tumour grade, proliferative activity and p53 immunoreactivity of tumour cells. APMIS 2007;115:1194-1205.
16. Di Carlo A, Terracciano D, Mariano A, Macchia V. Urinary gelatinase activities (matrix metalloproteinase 2 and 9) in human bladder tumors. Oncol Rep 2006;15:1321-1326.
17. Eissa S, Ali-Labib R, Swellam M, Bassiony M, Tash F, El-Zayat TM. Noninvasive diagnosis of bladder cancer by detection of matrix metalloproteinases (MMP-2 and MMP-9) and their inhibitor (TIPM-2) in urine. Eur Urol 2007;52:1388-1397.
18. Kanayama H, Yokota K, Kurokawa Y, Murakami Y, Nishitani M, Kagawa S. Prognostic values of matrix metalloproteinase-2 and tissue inhibitors of metalloproteinase-2 expression in bladder carcinoma. Cancer 1998;82:1359-1366.
19. Kexin XU, Shukun H, Zhijun DU. Prognostic value of matrix metalloproteinase-2 and tissue inhibitors of metalloproteinase-2 in bladder carcinoma. Chin Med J 2002;115:743-745.