ARTICLE ORIGINAL

Intérêt pronostique de l'étude de l'expression du CD44 dans les tumeurs urothéliales de la vessie

Prognostic value of CD44s expression in urothelial bladder tumors

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RÉSUMÉ

Introduction: Les tumeurs urothéliales(TU) de la vessie constituent un groupe hétérogène de tumeurs d'évolution imprévisible avec un important risque de récidive et de progression. Cette agressivité biologique traduit tout l'intérêt accordé aux études fondamentales sur la carcinogénèse vésicale et en particulier, à l'étude du CD44s comme marqueur de l'adhésion cellulaire et des cellules souches cancéreuses.

Objectif : Le but de ce travail était d'étudier l'expression phénotypique et l'impact pronostique du CD44s.

Méthodes : Etude rétrospective portant sur 38 cas de TU de la vessie. L'étude du CD44s a été réalisée par méthode immunohistochimique : seule une immunoréactivité membranaire est considérée positive. Cette expression a été étudiée en fonction des paramètres épidémiologiques et pronostiques universels des tumeurs urothéliales de la vessie.

Résultats : L'âge moyen des patients était de 61,24 ans avec un ratio homme /femme de 18/1. Les tumeurs étaient dans 87% des cas CD44s positives. Aucune association statistiquement significative n'a été retrouvée entre l'expression du CD44 et les paramètres : âge, sexe, taille tumorale, multifocalité, stade, récidive et progression tumorale. La perte d'expression du CD44 était cependant associée à un haut grade. La distribution de l'immunomarquage était par ailleurs corrélée à la multifocalité, au grade et au stade.

Conclusion : L'étude de l'expression du CD44 dans les tumeurs de la vessie révèle son intérêt pronostique et souligne une hétérogénéité phénotypique liée d'une part à l'altération de ses fonctions d'adhésion cellulaire et d'autre part à ses propriétés de marqueurs des cellules souches cancéreuses.

Mot clés : tumeurs urothéliales, vessie, immunohistochimie, CD44, grade, pronostic

SUMMARY

Background: Urothelial bladder carcinoma (UBC) includes a large group of malignancies with a variable clinical behavior. Despite remarkable developments in recognition of bladder carcinogenesis and prognostic factors, the recurrence rate is still high. Thus, identification of novel biomarkers involved in tumor cell invasion and metastatic dissemination is a constant challenge.

Aim: The aim of the study was to assess the prognostic impact of CD44 standard (CD44s) expression in UBT.

Methods: We assessed the immunohistochemical expression of CD44 in 38 samples of endoscopically resected UBT. Only membranous staining was considered positive. We analyzed topographic distribution of CD44s staining. Correlation of CD44s expression, clinicopathological features and disease progression was analyzed by Chi2 and Fisher tests. Kaplan-Meier analysis was used to investigate CD44s prognostic value.

Results: The mean age of patients was 61,24 years with male to female ratio of 18/1. CD44s expression was positive in 33 cases (87%). There was no significant correlation between CD44 expression and the parameters: age, gender, tumor size, focality, tumor site, stage, recurrence and tumor progression. CD44s loss of expression is, nevertheless, correlated with a high tumor grade. Topographic distribution of CD44s staining was associated with focality, grade and stage. Basal/parabasal staining expanded to the tumor layers in homogeneous "laminate" pattern used to be of better prognosis, compared to the heterogeneous "islets" or "dispersed" pattern .

Conclusions: Our results highlighted the prognostic value of CD44 expression in UBT. Focusing especially on staining pattern offers a better understanding of bladder carcinogenesis mechanisms.

Key words: urothelial cancer, bladder, immunohistochemistry, CD44, grade, prognosis

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INTRODUCTION

Urothelial bladder tumor (UBT) is an intricate malignancy with variable clinical behavior and a high risk of recurrence and tumor progression for both non-invasive and invasive urothelial carcinomas (1).

Despite development in diagnostic and prognostic markers, UBT remains a challenge in the oncology field, representing an ideal candidate for research on bladder carcinogenesis and novel prognostic biomarkers that could identify patients at increased risk of recurrence and progression.

Studies used to focus on clonal genetic and epigenetic alterations involved in tumor heterogeneity. However, according to recent data, heterogeneity among the entire tumor cells exist as a consequence of asymmetric division of cancer stem cells (CSCs) within the tumor mass, and all other cells comprising the tumor bulk are the result of differentiated CSC. In this perspective, the epithelial-mesenchymal transition (EMT) process plays a major role in cancer progression and metastasis (2,3).

CD44 (and its standard isoform (CD44s)) is a cell surface adhesion molecule involved in cell-cell and cell-matrix interactions. Recent evidence has strengthened the potential role of CD44 in CSC and EMT. It is involved in the progression and invasion of several human malignancies such as breast and colorectal cancers (4, 5). Some investigations have focused on the prognostic value of CD44 in UBT, however provided inconclusive or contradictory results. In this study, we aimed to assess the prognostic impact of CD44s expression in UBT.

METHODS

Patients and clinocopathological data:

A total of 38 patients with primary UBT underwent endoscopic tumor resection at our hospital, from 2009 to 2013. We retrospectively analyzed the clinical and pathological data of the patients. Note that, patient confidentiality was preserved and informed consents were obtained from all participants.

The evaluation variables were age, gender, tumor size, focality, reccurence and tumor progression. Histological subtype and stage were classified according to the 2016 World Health Organization (WHO) classification of urothelial tract tumors and the 2017 TNM classification (6, 7). Grading was established according to 1973 WHO criteria (8).

Samples with necrotic tissue or burnt necrosis due to electrocoagulation were excluded from the study. Patients with tumor recurrence that were previously treated by Bacillus Calmette-Guerin (BCG) were also elbowed out of the study.

Immunohistochemical assessment of CD44:

Selected antigens were detected immunohistochemically, using monoclonal mouse IgG1 reactive antibodies that recognizes CD44s (Leica, clone DF1485, diluted 1:50). Four-micrometer sections were made from the formalin-fixed, paraffin-embedded blocks, which were then deparaffinized in xylene and rehydrated in a graded series of alcohol solutions. For antigen retrieval, paraffin tissue sections were cooked for 2-5min with 10 mM sodium citrate buffer, pH 6.0, at a sub-boiling temperature for 15 min and cooled for 20 min at room temperature. The sections were treated with 3% hydrogen peroxide in methanol and then incubated overnight at 4°C with monoclonal primary antibodies diluted in 1% bovine serum albumin. After washing, the primary antibody was detected with appropriate secondary antibody for 30 minutes at 37°C. Following washes, slides were incubated in avidin-biotin complex for 20 minutes at 37°C and visualized using diaminobenzidin as the chromogen. Afterwards, the slides were briefly counterstained with hematoxylin, dehydrated and mounted. Immunostained samples were evaluated by a pathologist. Immunolabeling was considered positive when tumor cells showed cytoplasmic membrane staining. The conjunctive cells of the subepithelial connective tissue were considered as positive control stain.

The topographic pattern of CD44s staining was assessed depending on the staining levels in tumor layers and its architecture. It was divided into four categories:

- Basal /parabasal: positive tumor cells were situated in the basal and parabasal tumor lining)

-Basal /parabasal with homogeneous expanded staining towards tumor layers, in a “laminate” mode.

-Basal /parabasal with heterogeneous expanded staining towards tumor layers, in an “islets” mode: accentuated expression in uni or multicellular clusters

- "Patchy" pattern: alternating positive and negative staining zones

Statistical Methods:

Based on the collected data, we conducted a statistical study using EpiInfo software. Quantitative and qualitative data were expressed using averages and percent, respectively. Pearson's χ2 and Fisher tests were used to investigate the correlations between CD44s expression, its topographic distribution, clinical, histopathological data and progression. P<0.05 was considered to indicate a statistically significant difference.

Recurrence-free survival curves were plotted using the Kaplan-Meier method and analyzed using the log-rank test.

RESULTS

Clinicopathological characteristics:

Our study group included 36 males (95%) and 2 female patients (5%) (sex ratio M/F:18/1), ranging from 33 to 85 years of age (average = 61.24 years).

Patient and tumor characteristics are summarized in Table 1.

Progressive features:

Recurrence:

Median recurrence-free survival (RFS) was 13.5 months [2-54 months]. Tumor recurrence was reported in 15 patients (39.5%) (Table1). It was observed in 26% of patients with pTa tumors (5 /19), 46% with pT1 tumors (6 /13) and in 67% of patients with pT2 tumors (4/6).

In association to histological grade, recurrence was noticed in 23% of patients with G1 tumors (3/13), in 42% with G2 tumors (5/12) and in 54% of patients with G3 tumors (7 /13).

Tumor progression:

Median progression-free survival (PFS) was 14.3 months [3- 54 months]. Tumor progression was noted in 5 patients (13%) with pTa/ pT1 tumors and 2 with pT2 tumors (Table 1).

Table 1. Patient and tumor characteristics of the 38 patients

Variables	No of cases (%)
Age	
≤60 ans	21 (55.3%)
>60 ans	17 (44.7%)
Gender	
M	36 (95%)
F	5 (5%)
Focality	
Unifocal	21 (55%)
Multifocal (2-7)	17 (45%)
Size	
<3 cm	15 (39%)
≥ 3 cm	23 (61%)
Location	
Neck and trigone	16 (40%)
Urethral orifice	10 (25%)
Side face	7 (17,5%)
Base	3 (7,5%)
Dome	2 (5%)
Prostatic urethra	2 (5%)
Growth pattern	
Papillary	38 (100%)
Solid	0
[a]Grade	
G1	13 (34%)
G2	12 (32%)
G3	13 (34%)
[b]Stage	
pTa	19 (51%)
pT1a/b	7/6 (33%)
pT2	6 (16%)
[c]Infiltrating UC	
Conventional type	14/19 (73.7%)
With squamous diff	3/19 (15.8%)
With glandular diff	2/19 (10.5%)

In association to histological grade, 2 patients had progressed with respectively G2 and G3 tumors, and one patient with G1 tumor has progressed.

Immunohistochemistry:

A total of 33 patients (87%) were CD44s positive whereas 5cases (13%) were CD44s negative. Topographic distribution of CD44s expression was assessed as follows: five patients (15.1%) with basal/parabasal staining (Figure 1), 15 patients (45.5%) with basal /parabasal and expanded staining in a "laminate" mode (Figure 2A), 6 patients (18.2%) with basal /parabasal and expanded

staining in an “islets” mode (Figure 2B) and 7 patients (21.2%) with “patchy” staining (Figure 3).

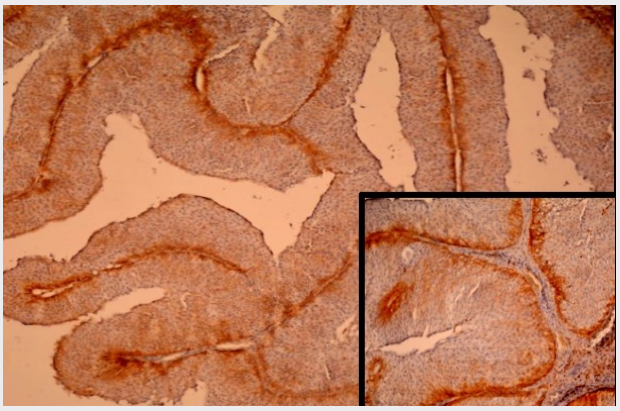

Figure 1. Basal/parabasal CD44s expression in papillary urothelial tumor (IHCx100). In cart: IHCx200

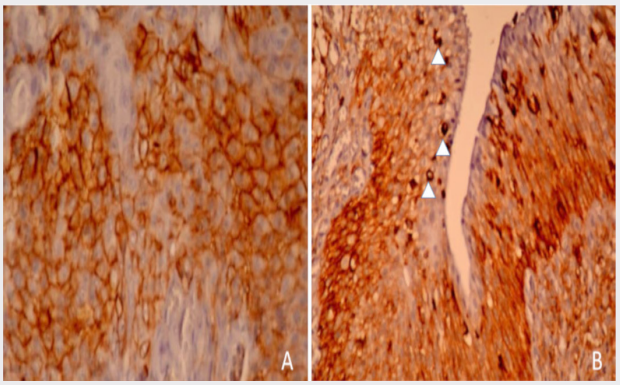

Figure 2. Expansion of CD44s staining towards tumor surface in « laminate » (A) and « islets » mode (arrowheads) (B) (CD44s x 200)

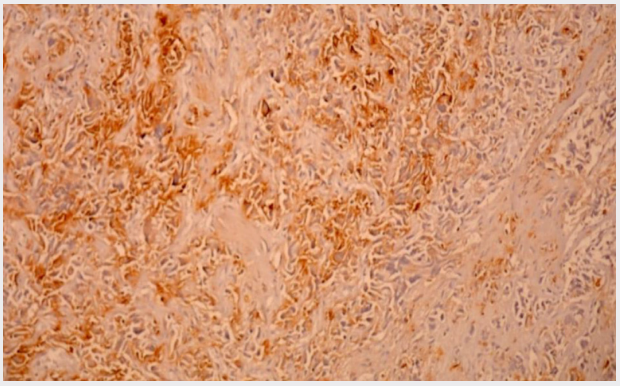

Figure 3. « Dispersed» CD44s staininig within carcinomatous area in invasive urothelial tumor (IHCx200)

Correlation between CD44s expression and clinicopathological and progression variables (Table 2):

There was no correlation between CD44s expression and the clinicopathological factors: age, gender, tumor size, focality, tumor localization and stages. However, loss of CD44s expression was correlated with histological grade. CD44s decreases in high grade tumors: all patients with G1 tumors were CD44s+; 83.3% of patients with G2 were CD44s+ and only 77% in G3 (p=0.03).

Note that topographic distribution of CD44s expression showed no association with age, gender, tumor size and site. Nevertheless, there was significant difference between the distribution of CD44s expression and the variables: grade and stage.

Concerning focality, the basal/parabasal staining with expansion was significantly more frequent than « dispersed » topography in multifocal tumors (p= 0.02).

Regarding histological grading, no patient with G1/2 tumors had « dispersed » staining, whereas in G3 patient group, 7 (70%) had this pattern of stain and none had basal/parabasal staining. In our study, half of CD44s+ pTa/pT1 patients had “laminate” staining, and only invasive UBT (pT1 and pT2) had “patchy” CD44s expression (p=0.001).

CD44s staining and its topographic distribution showed no association with recurrence and tumor progression (Table 2). Nevertheless, some facts deserve to be highlighted:

- Tumor recurrence occurred in one patient (20%) among the 5 that were CD44s- and in 14 patients (42%) among the 33 that were CD44s+.
- For CD44s+ patients, one-year RFS was at 67% compared to 80% for those CD44s-, with no significant difference (p = 0.72).
- Patients with tumor recurrence had predominant CD44s staining (71.5%) in « islets » expansion and in “patchy” pattern.
- Among the 33 patients with no progressive disease, 84.8% had CD44s + tumors but on the other hand, all 5 patients with progressive disease had also CD44s+ tumors.
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Table 2. Statistical analysis-correlations between CD44s expression and prognostic parameters in the 38 patients

Variables	CD44s Staining N (%)		p	Topographic distribution of CD44s expression N (%)				p
	Neg	Pos		B+SB	LExp	IExp	DExp	
Age (years)	70.4	59.8	0.11	57	52	61	58	0.6
Gender								
M	5 (13.9)	31 (86.1)	0.85	5 (16.1)	14 (45.2)	6 (19.4)	6 (19.3)	0.54
F	0	2 (100)		0	1 (50)	0	1 (50)	
Focality								
Unifocal	1 (4.8)	20 (95.2)	0.06	5 (25)	5 (25)	3 (15)	7 (35)	**0.02**
Multifocal	4 (23.5)	13 (76.5)		0	10 (77)	3 (23)	0	
Size								
< 3 cm	2 (13.4)	13 (86.6)	0.35	4 (30.7)	8 (61.6)	0	1 (7.7)	0.57
≥ 3 cm	3 (13)	20 (87)		1 (5)	7 (35)	6 (30)	6 (30)	
Location								
Neck/trigone	2 (12.5)	14 (87.5)	0.91	3 (21.5)	4 (28.5)	4 (28.5)	3 (21 .5)	0.97
Others	3 (13.6)	19 (86.4)		2 (10.5)	11(58)	2 (10.5)	4 (21)	
[a]Grade								
G1	0	13 (100)	**0.03**	3 (23)	9 (70)	1 (7)	0	**0.01**
G2	2 (16.7)	10 (83.3)		2 (20)	5 (50)	3 (30)	0	
G3	3 (23)	10 (77)		0	1 (10)	2 (20)	7 (70)	
[b]Stage								
pTa	3 (15.8)	16 (84.2)	0.8	3 (18.8)	8 (50)	5(31.2)	0	**0.001**
pT1	1 (7.7)	12 (92.3)		2 (16.7)	6 (50)	1 (8.3)	3 (25)	
pT2	1 (16.7)	5 (83.3)		0	1 (20)	0	4(80)	
Recurrence								
No	4 (17.4)	19 (82.6)	0.12	4 (21)	12 (63.2)	0	3 (15.8)	0.43
Yes	1 (6.6)	14 (93.4)		1 (7.2)	3 (21.5)	6 (43)	4 (28.3)	
Tumor prog								
No	5 (15.2)	28 (84.8)	0.57	5 (17.9)	10 (35.8)	8 (28.5)	5 (17.8)	0.26
Yes	0	5 (100)		0	2 (40)	1 (20)	2 (40)	

DISCUSSION

CD44 is a type I transmembrane glycoprotein receptor that binds primarily to the extracellular glycosaminoglycan hyaluronan. This protein is also known as a cellular adhesion molecule and has been linked to diverse effects including cellular adhesion, migration and invasion, which are crucial in cancer progression [4,5].

In urothelial bladder tumors, studies used to focus on the % of CD44s positive cells and the score of staining (9, 10). In this study we aimed to investigate CD44s expression in 38 patients with endoscopically resected UBT and focus on the topographic distribution pattern. 87% of the selected cases were CD44s positive, which is consistent with the literature data with variable incidence ranging from ranges from 28% to 91%. This CD44s expression variability could be related to the structural changes of the secondary protein by alternative splicing, to the lack of standardization of the CD44s interpretation criteria and to the different clonal properties of the antibodies used in the different studies (10-13).

We did not observe any correlation between CD44s expression and the variables: age, gender, tumor size, focality, tumor localization, stages, recurrence and tumor progression. These findings are consistent with those of many published studies [8, 16].

However, our data revealed that the loss of CD44s expression was significantly correlated with grade (p=0.03). In UBT, the prognostic value of CD44s expression is still controversial. Molecular mechanisms are not yet well established and CD44s seems to be a key link for tumor promotion/suppression (15-18). The grade is correlated with tumor aggressiveness and represents an independent predictive factor of survival and parietal invasion (14-15). In our study, all patients with G1 tumors were CD44s+ versus 83% in G2 and 77% in G3 (p=0.03). These results are in accordance with those of several studies (19-20). According to Desai S et al, a significant relationship was found between grade and CD44s expression (20).

In the Ross study of 44 UBT, CD44s + expression was 61% in grade 1 tumors versus 30% in G3 tumors (p=0.001) (21). Further studies demonstrated that in contrast, high-grade tumors were associated with a high rate of CD44s expression (9).

Our data revealed no correlation between CD44s expression and progression variables.. Nevertheless, patients with CD44s+ expression are more likely to develop more progressive disease. In fact, for CD44s + patients, one-year RFS and PFS were inferior to those CD44s- (67% versus 80% ; p = 0.72 and 55% versus 70% ; p = 0.6 respectively). Furthemore, tumor recurrence occurred in 20% of patients with CD44s- tumors and in 42% in those with CD44s+. These finding have also been reported by Keymoosi et al (15).

In the study of Stavropoulos et al, PFS was 32.3 months for CD44s+ patients and 16.4 months for CD44s-patients without significant difference (22). All these inconsistent findings make it difficult to assess the prognostic value of CD44s expression in UBT.

In our series, we especially focused on the CD44s topographic distribution, as the pattern of stain can be closely related to the bladder carcinogenesis.

Topographic staining was basal/parabasal in 15.1% of patients, with «laminate » expansion in 45.5% of patients and with « islets » one in 18.2% of patients. “Patchy” staining pattern was observed in 21.2% of patients. CD44s topographic staining was not associated with age, gender, tumor size, localization, recurrence and tumor progression. We observed a significant difference between topographic distribution of CD44s expression and: focality (p=0.02), grade (p=0.01) and stage (p=0.001).

Interestingly, CD44s topographic pattern of stain had a prognostic impact. Patients with either exclusive basal/ parabasal positivity or associated with « laminate » expansion to the epithelium surface had better prognosis than those harboring “islets” expansion or “dispersed” pattern.

Variant isoform of CD44 (CD44v) is generated by alternative splicing of CD44 gene exons (9). The standard isoform (CD44s) is the most common and the smallest variant which is present on the surface of a wide variety of epithelial and mesenchymal cells, especially in the basal and parabasal layers of the normal bladder urothelium (10).

This study grows out from the active and emerging researches on CD44 crucial role in the initiation, progression and recurrence of various malignancies. In fact, tumor cells expressing CD44 exhibit CSC and EMT cell-mediated properties, which could play a key role in favoring both tumor development and metastatic dissemination (10, 23-25).

CD44s topographic distribution would be closely related to bladder carcinogenesis: it begins in papillary tumors pTaG1 with a basal / parabasal expression, expanding gradually to the surface in “laminate” mode. This homogeneous expression in low grade carcinomas would become more heterogeneous in high-grade ones, with « islets » and « dispersed » expression (20-22). This expression heterogeneity would be linked to a gene dysregulation, causing alteration in cell adhesion properties in favor of oncogenic actions (26,27).

Our findings should be however reported with caution considering the small sample sizes. Further studies are needed to assess definitively the prognostic value of CD44s in UBT and to define criteria for immunohistochemical staining.

CONCLUSIONS

Our results demonstrated that loss of CD44s is significantly correlated to grade and highlighted the impact of topographic distribution of CD44s expression. Our findings suggest that a basal/parabasal staining with “laminate” expansion towards tumor surface was of better prognosis than those having “islets” extension or “patchy dispersed” pattern.

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