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E-cadherin/CD44 immunophenotype in the epithelial–mesenchymal transition of bladder urothelial carcinomas

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Abstract

The alteration of epithelial stability, which includes changes in the expression of E-cadherin and CD44, is one of the complex biomolecular mechanisms involved in the tumoral epithelial–mesenchymal transition (EMT) process. We followed the E-cadherin/CD44 immunophenotype by single and double detections in 25 cases of bladder urothelial carcinomas. Our study investigated simultaneously the differences in expression of the two markers, in different tumoral compartments, according to the prognostic parameters of the lesions. The study indicated significant differences in the expression of E-cadherin in relation to tumor grade, depth of invasion, tumor stage and Ki-67 proliferation index (PI), both intratumoral and at the advancing edge. For CD44, expression differences were found between the tumor grades in intratumoral sites, while for both intratumoral and advancing edge compartments the differences occurred for the depth of tumor invasion, tumor stage and Ki-67 PI. The only differences in the expression of the two markers in relation with the presence of lymph node metastasis were for E-cadherin at the advancing edge. In this study, the intratumoral E-caderin-/CD44- immunophenotype, respectively E-caderin-/CD44+ at the advancing edge were associated with the tumor aggressiveness analyzed parameters. The maintenance of CD44 expression at the advancing edge represents a negative prognostic factor for bladder urothelial carcinoma and supports the implication of EMT process, through the existence at this level of a cell population with particular properties.

Keywords: CD44, E-cadherin, bladder carcinoma, epithelial–mesenchymal transition.

Introduction

Urothelial carcinoma is a common lesion of the urinary tract, representing over 90% of bladder malignancies [1, 2]. It ranks on the 7th place in European males, accounting for over 35 000 of deaths each year [3]. Biomolecular mechanisms underlying urothelial carcinogenesis have been extensively studied aiming at identifying biomarkers predictive for tumors' behavior. In this view, epithelial–mesenchymal transition (EMT) is a process in which epithelial cells acquire a mesenchymal phenotype, playing a key role in tumor progression, respectively in invasion and metastasis [4–8]. In this context, the altered expression of E-cadherin and CD44 molecules involved in epithelial stability may represent targets for investigating the EMT process.

The majority of published studies conducted on malignant tumors of different localizations, including urothelial localization, indicate that E-cadherin is the most important mediator of intercellular adhesion, and its altered expression associates with the risk of invasion and metastasis [9–12]. During the EMT process, an essential time point is the so-called "cadherin switch", in which E-cadherin is replaced by N-cadherin [13].

On the other hand, epithelial integrity is maintained by cell surface proteins that are connected with the intercellular matrix and the underlying connective tissue. Among these, CD44 is the main receptor for hyaluronic acid, which mediates the interaction between epithelium and mesenchymal elements and can promote the EMT process [10, 14, 15].

The majority of the studies analyzing E-cadherin and CD44 were based on different groups of patients, and indicated an inverse correlation between the expression of the two proteins with the tumoral grade and stage of bladder urothelial carcinomas [16–19]. Other authors support the prognostic value of E-caderin just in T2/T3 carcinomas [16]. Hong *et al.* [20], analyzing the expression of these proteins on a common group of patients with urothelial carcinomas, indicated a loss of E-cadherin expression and the conservation of CD44 expression in poorly differentiated carcinomas. Several recent studies on oral, esophagian, thyroidian and breast carcinomas indicated that the conservation of CD44 expression constitutes a negative prognostic factor [21–24].

In order to clarify these discordances and in the context of E-cadherin and CD44 involvement in the EMT process,

in the present study we have followed this markers' immunophenotype in bladder urothelial carcinomas, at the level of different tumoral compartments and depending on some of the prognostic parameters.

Materials and Methods

The study included a total of 25 patients hospitalized in the Clinic of Urology of Emergency County Hospital of Craiova, Romania, in the period 2013–2014. Biological material was represented by cystectomy specimens. The histopathological diagnosis was performed in the Laboratory of Pathology, where the specimens were fixed in 10% buffered neutral formalin, processed for paraffin embedding and Hematoxylin–Eosin staining. Clinico-morphological parameters investigated were: age, gender, degree of differentiation, tumor topography (intratumoral *versus* the advancing edge), the degree of proliferation (Ki-67 index), depth of invasion, lymph node status, pTNM stage. For the assessment of the lesions, we utilized the *WHO* 2004 staging system [25]. In this study were included only primary papillary urothelial carcinomas, which had no distant metastases.

The study was approved by the local ethical committee, and a written informed consent was obtained from all the patients. It is of interest to mention that in Romania there is no National Register for cancers.

In order to assess the aggressiveness of these tumors, we investigated E-cadherin/CD44 immunophenotype in relation to the morpho-clinical parameters mentioned above. For immunohistochemical analysis, we used a panel of antibodies, as shown in the table below (Table 1).

Table 1 – ***The antibodies used in the study***

Antibody	Clone	Dilution	Pretreatment	External positive control
E-cadherin	Mouse, NCH 38, Dako	1:50	Microwaving in citrate buffer, pH 6	Mammary gland
CD44	Mouse, DF 1485, Dako	1:30	Microwaving in citrate buffer, pH 6	Spleen
Ki-67	Rabbit, Polyclonal, Thermo Scientific	1:100	Microwaving in citrate buffer, pH 6	Breast carcinoma

For immunohistochemistry on selected serial sections, after antigen retrieval, endogenous enzyme blocking, and unspecific sites' blocking, the sections were incubated overnight at 4^0C with the E-cadherin and CD44 monoclonal antibodies. The next day, the sections were incubated with biotinylated species-specific secondary antibodies, which were later on amplified with the LSAB 2 System–HRP (DAKO, Redox, Bucharest, Romania, code K0675) and visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dako, code 3467). For double detections of E-cadherin/Ki-67 and respectively CD44/ Ki-67, the two protocols were performed sequentially, the amplifications being made with LSAB2 HRP and respectively LSAB 2 System–AP (Dako, Redox, code K0674), and the detections made with DAB and respectively Vulcan Fast Red (Biocare Medical, Redox, code FR805S). Between the procedures, a Biotin-blocking step was intercalated (Dako, Redox). Finally, the slides were counterstained with Hematoxylin and coversliped with DPX (Fluka, Redox) (for single DAB-based stainings), or with a glycerol-based mounting medium (Dako, Redox) (for double stainings).

The quantification of the reactions was performed on double stainings by IOD (integrated optical density), which is the product of the area of a region of interest (ROI) and the average density of the pixels that compose the region. For each image, we summed the IOD values of all the regions of interest. Finally, we obtained an IOD value to obtain a specific indicator for each case. To quantify the Ki-67 immunoexpression, we calculated the proliferation index (PI), by dividing the number of positive tumor nuclei to the total number of tumor nuclei counted in the 40× microscopic field, and counting at least 2000 nuclei per case [26]. Two observers (AS and DP) performed the evaluation of the staining. We used external positive controls (Table 1) and respectively negative controls, by omitting the primary antibodies. The acquisition of the images was done on a Nikon Eclipse E600 microscope and with the software package Lucia 5. The image analysis was done with Image ProPlus 7 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

For the statistical analysis, it was created an electronic database and the results were compared using the Student's *t*-test (SPSS, Inc., Chicago, IL, USA). Also, we used one-way ANOVA test to assess the differences between more than two independent groups. All central tendencies were reported as average ± standard deviation (SD). Results were considered significant for *p*-values <0.05.

Results

From the total of 25 patients, the analysis of clinico-morphological parameters indicated an average age at diagnosis of 62.5 years, with the predominance of male patients (19 cases) (Table 2). Most of the lesions presented a low tumoral grade (14 cases), invasion into the lamina propria (10 cases), without lymph node metastases (23 cases), the majority being classified in the pTNM stage I of disease (10 cases) (Table 2). The average value of Ki-67 proliferation index in intratumoral and advancing edge areas was of 22.3±10.7, respectively of 30.3±15.1, a difference which was statistically significant (p=0.037, Student's *t*-test) (Table 2).

Table 2 – ***Cases distribution depending on the investigated parameters***

Parameter	Variable (No.)
Age [years]	<50 = 2
	50–70 = 21
	>70 = 2
Gender	Males = 19
	Females = 6
Differentiation degree	LG = 14
	HG = 11
Depth of invasion	T1 = 10
	T2 = 8
	T3 = 7

Parameter	Variable (No.)
Lymph node metastasis	N0 = 22
	N1 = 3
Stage	I = 10
	II = 8
	III = 4
	IV = 3
Proliferation index (Ki-67)	IT = 22.3±10.7
	AE = 30.3±15.1

LG: Low grade; HG: High grade; IT: Intratumoral; AE: Advancing edge.

E-cadherin immunostaining

E-cadherin immunostaining was identified on the cells' membranes on intratumoral localizations in 88% of cases, and at the level of the advancing edge in 52% of cases, negative cases belonging mostly to high-grade carcinomas with invasion at least in muscularis propria and lymph node metastases. The reactions were observed mainly in the superficial layers of the tumor papillae and decreased towards the intermediate and basal layers (Figure 1, A–D).

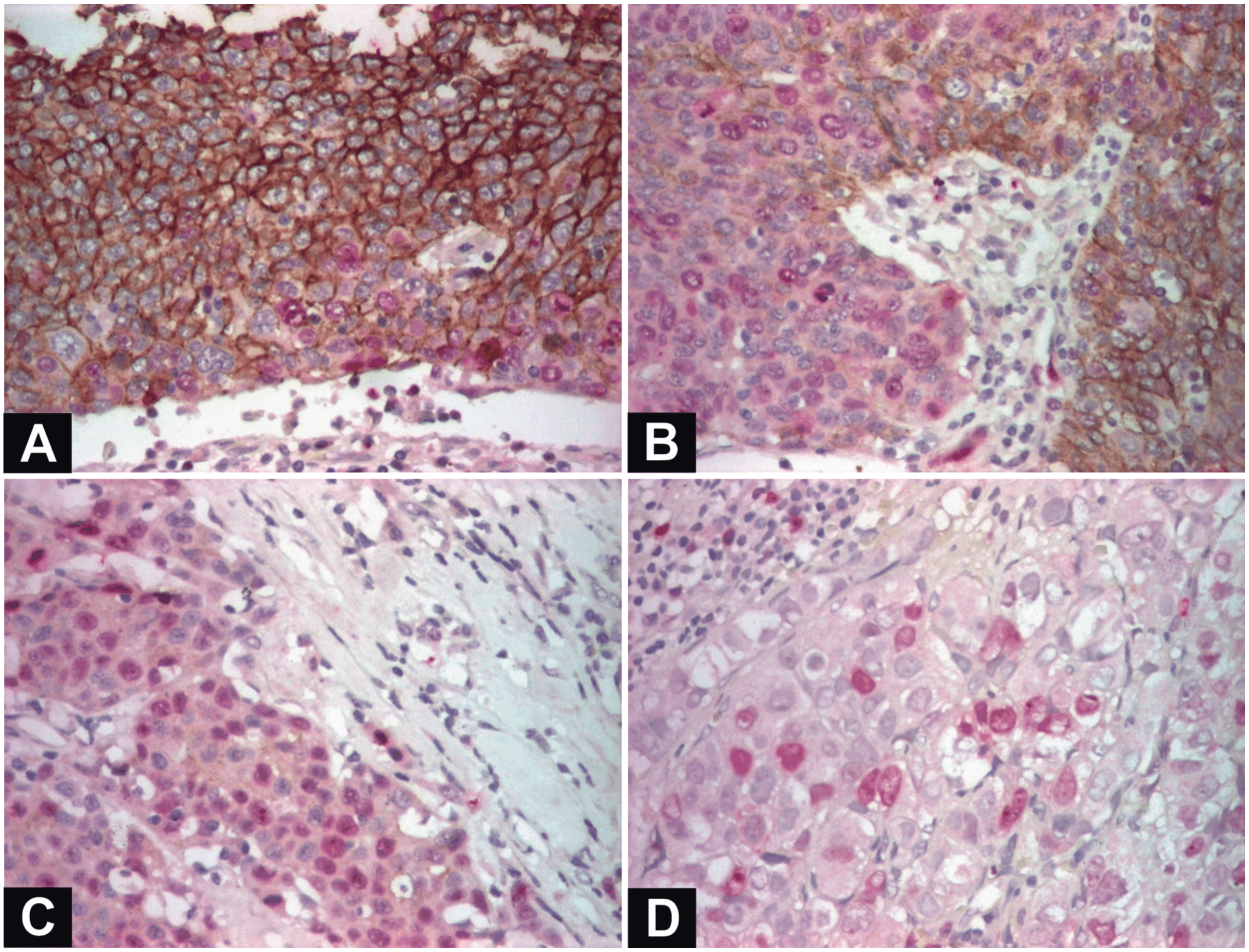

Figure 1 – ***Urothelial carcinoma, E-cadherin/Ki-67 immunostaining, 200×: Low-grade, intratumoral (A); High-grade, intratumoral (B); High-grade, advancing edge (C); Lymph node metastasis (D).***

The analysis of E-cadherin IOD indicated intratumoral significantly higher values ($247\times10^5\pm177\times10^5$), compared to the advancing edge ($25\times10^5\pm28\times10^5$; p=0.000, Student's t-test), a compartment where the marker's expression decreased, regardless of the clinicopathological parameters analyzed. Statistical analysis also indicated significant differences in the expression of E-cadherin in relation to tumor grade, the IOD values being significantly higher in low-grade carcinomas compared to high-grade lesions, both intratumoral (p=0.006, Student's t-test) and at the advancing edge (p=0.021, Student's t-test) (Figure 2A).

In relation to the depth of invasion, IOD values were significantly higher in pT1 carcinomas compared with pT2-T3 lesions in both intratumoral (p=0.000, ANOVA test) and at the advancing edge (p=0.000, ANOVA test) (Figure 2B). In the case of urothelial carcinomas with lymph node metastases, the IOD E-cadherin values presented significant lower differences at the advancing edge (p=0.000, Student's t-test), compared to cases without metastases, the differences being insignificant at the intratumoral level (p=0.070, Student's t-test) (Figure 2C). E-cadherin expression analysis in relation to the tumor stage indicated significantly higher values in case of stage I carcinomas compared to the stages II–IV lesions, both intratumoral (p=0.000, ANOVA test) and at advancing edge (p=0.000, ANOVA test) (Figure 2D).

Pearson's test indicated a negative linear correlation between the intratumoral E-cadherin IOD and Ki-67 PI values, [$r(23)$=-0.640, p=0.001].

The same statistical relation was found between the two parameters at the advancing edge, [$r(23)$=-0.584, p=0.002].

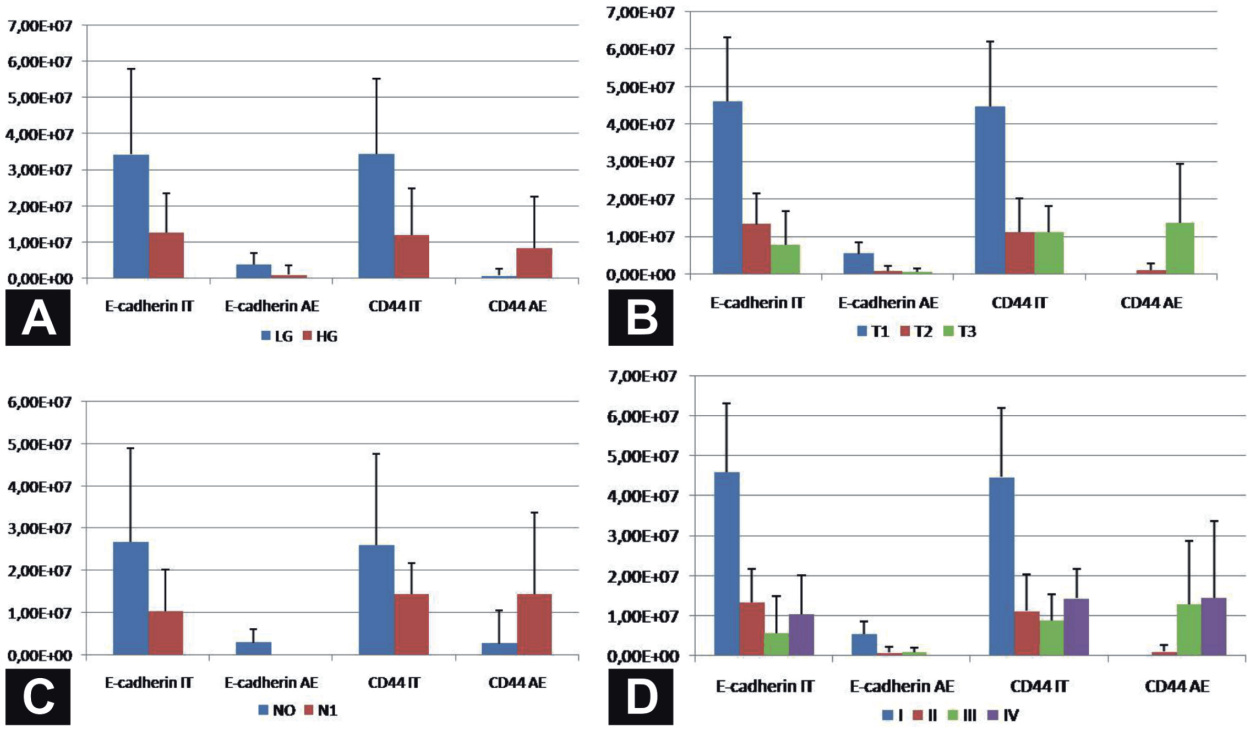

Figure 2 – ***Distribution of the E-cadherin and CD44 mean IOD values, intratumoral and at advancing edge in relation to tumor grade (A), depth of invasion (B), presence of lymph node metastasis (C), tumor stage (D).***

CD44 immunoreaction

CD44 immunoreaction was identified in the membrane, intratumorally in 92% of cases, the reaction being absent in two high-grade carcinomas. The staining was present mainly in the basal layer, diminishing towards the intermediate and superficial ones. In the advancing edge, the CD44 reaction was observed in 64% of cases. Positive carcinomas were mostly of high grade, with at least muscularis propria invasion and lymph node metastases. In addition, the reaction was noticed in some stromal elements as lymphocytes, fibroblasts, macrophages (Figure 3, A–D).

The analysis of CD44 IOD on the entire group indicated significantly higher intratumoral values ($245\times10^5\pm 203\times10^5$), compared to advancing edge ($40\times10^5\pm103\times10^5$; p=0.000, Student's t-test), regardless of clinicopathological analyzed parameters.

In relation to the tumor grade, the differences between CD44 IOD values were significant only at intratumoral level (p=0.003, Student's t-test), these being higher in low-grade carcinomas compared with high-grade lesions (Figure 2A). In relation to the depth of invasion, IOD values were significantly higher in pT1 carcinomas compared with pT2-T3 lesions in both intratumoral (p=0.000, ANOVA test) and at the advancing edge (p=0.007, ANOVA test) (Figure 2B). We did not find significant differences in CD44 IOD values in relation to the presence of lymph node metastasis, regardless of tumor compartment being analyzed (intratumoral, advancing edge) (p>0.05, ANOVA test) (Figure 2C). The analysis of intratumoral CD44 expression depending on tumor stage indicated significantly higher IOD values in stage I carcinomas compared to the stages II–IV (p=0.000, ANOVA test). At the advancing edge, IOD values were significantly higher in stage III–IV carcinomas compared with those in stage II (p=0.021, ANOVA test) (Figure 2D).

Pearson's test indicated a negative linear correlation between the intratumoral CD44 IOD and Ki-67 PI values, [r(23)=-0.693, p=0.000]. At the advancing edge, a direct correlation between the two parameters could be noted, [r(23)= 0.507, p=0.010].

In this study, we found no association of E-cadherin or CD44 expression with the ages or genders of patients. Regarding the expression of the two markers, Pearson's test indicated an intratumoral positive linear correlation, [r(23)=0.762, p=0.000]. On the contrary, at the advancing edge, the relation was negative and statistically insignificant, [r(23)=-0.312, p=0.129], the decreasing of E-cadherin expression, being accompanied by the maintenance of CD44 expression for 62% of the analyzed cases.

Discussion

Many studies in the literature place E-cadherin and CD44 in the center of the biomolecular systems that ensure the stability of epithelia, including urothelium [9–11, 17]. E-cadherin is a Ca^{2+}-dependent transmembrane receptor that mediates intercellular adhesion, whereas CD44 is the active component of this system through which normal and tumor cells interact with adjacent matrix [27, 28]. Also, CD44 has been shown to be involved in many other biomolecular processes such as angiogenesis, tissue healing, lymphocytes extravasation and activation, cell migration, including EMT process [15, 28–31].

Recent studies investigating E-cadherin and CD44 in malignant tumors with other sites, identified close functional connections between the two proteins, with a CD44-positive staining being an indicator of a negative prognostic [21–24]. The role of CD44 and its interaction with other adhesion molecules is not fully understood.

On cell cultures, Xu & Yu [32] proved the existence of a negative modulation induced by E-cadherin on the link between CD44 and hyaluronic acid. Another recent study indicated a CD44 inhibitory effect of E-cadherin expression and overexpression of N-cadherin, α-actin, vimentin, fibronectin and MT1-MMP, issues characteristic of the EMT process [15].

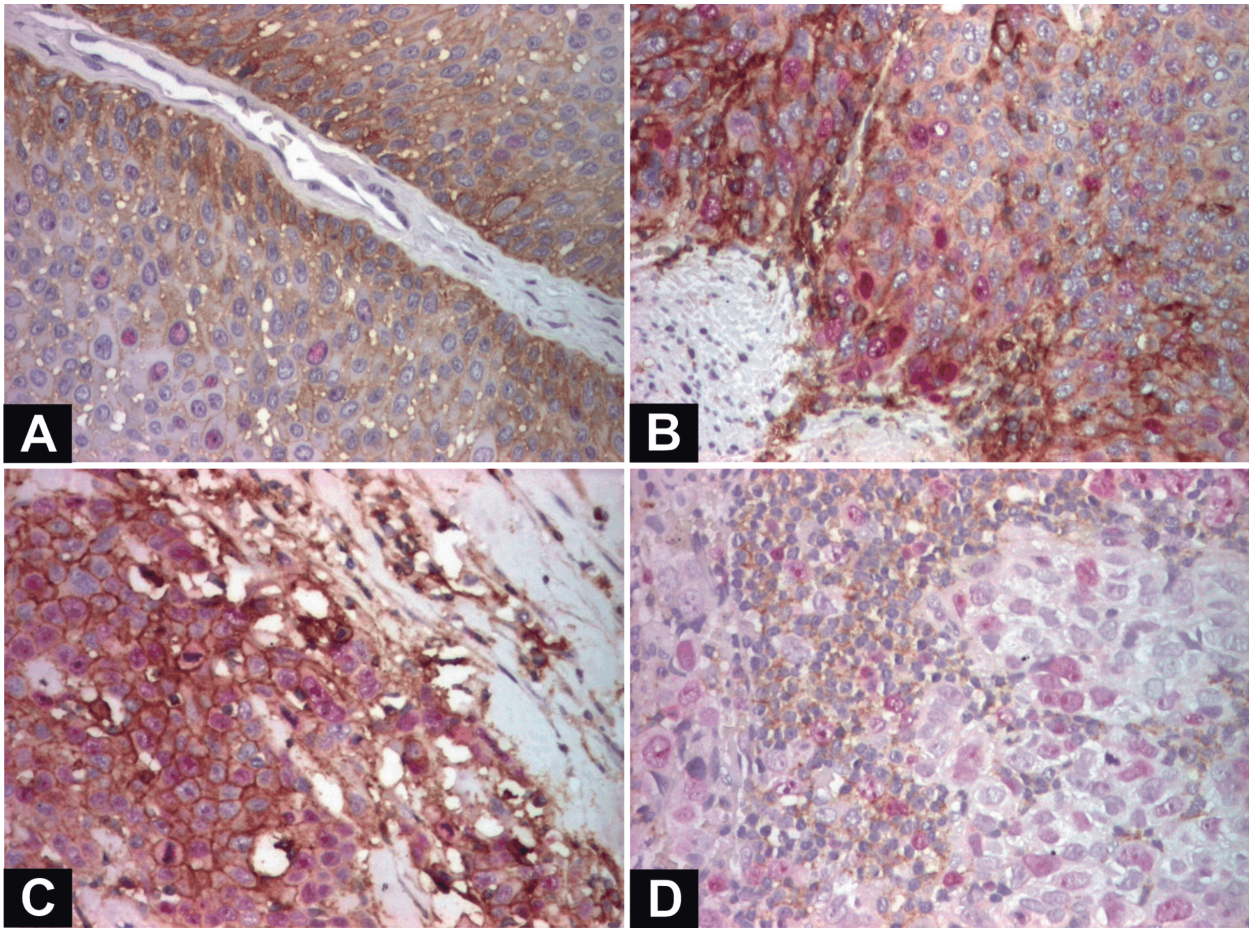

Figure 3 – ***Urothelial carcinoma, CD44/Ki-67 immunostaining, 200×: Low-grade, intratumoral (A); High-grade, intratumoral (B); High-grade, advancing edge (C); Lymph node metastasis (D).***

Most studies have analyzed individually the immunoexpression of E-cadherin and CD44, without looking at the combined expression patterns [9, 10, 16, 18, 19, 33–35]. Our study investigated simultaneously the differences in expression for the two markers according to various morpho-clinical parameters of the bladder urothelial carcinomas. We also investigated the expression of the two markers both intratumoral and at the advancing edge, where, according to the literature data, the EMT process can be observed [8].

In this study, we found significant differences in the E-cadherin IOD values depending on tumor degree, depth of invasion, tumor stage and Ki-67 PI at both intratumoral and advancing edge. In case of CD44, differences were significant at intratumoral level in relation with tumor degree, and in both compartments when the values were reported on the depth of tumor invasion, tumor stage and Ki-67 IP.

Similar studies in the literature that have investigated the expression of E-cadherin and CD44 in urothelial carcinomas indicated a correlation between the expression of the two proteins with the main prognostic parameters in urothelial carcinomas, such as tumor stage, risk of metastasis and sometimes tumor grade [9, 10, 17, 36].

In some more recent studies, it is indicated that E-cadherin expression is associated with tumor stage and tumor grade, considering it useful for assessing disease prognosis [33–34]. In 2006, Koksal *et al.* [37] argued that the aberrant E-cadherin expression can be identified in over 85% of bladder urothelial carcinoma at an advanced stage of disease. In the present study, we found a significant decrease of E-cadherin expression in high-grade carcinomas, with increased proliferation index, deep invasion and metastasis. The expression of E-cadherin at the advancing edge diminished, being absent in high-grade carcinomas, regardless of other analyzed parameters.

There are few studies regarding CD44 immunoexpression in bladder urothelial carcinomas. The literature data designates the CD44 positivity as associated with T1 carcinomas, while the negative expression seems to be characteristic for T2/T3 carcinomas [10]. On the groups of urothelial carcinomas analyzed, Kong *et al.* [19], in 2003, and Kuncová *et al.* [35], in 2007, indicated that CD44 immunoexpression decreases with the depth of invasion, respectively the heterogeneous appearance of the immunostaining in poorly differentiated tumors. In this study, CD44 expression decreased in high-grade carcinomas with deep invasion. For 64% of the cases, we found a conservation of the CD44 expression at the

advancing edge and these cases were high-grade carcinomas with variable invasion in bladder wall and metastasizing.

Regarding the role of the two proteins in the development of metastases there are different opinions. In 2001, Byrne *et al.* [36] found that an abnormal expression of E-cadherin is associated with tumor invasion and the presence of lymph node metastasis. Based on the literature data and their own research, Gontero *et al.* [38] argues that in cases of urothelial carcinoma E-cadherin is useful for assessing the risk of metastasis, whereas the results are inconclusive for CD44. In our study, the only differences in the expression of the two markers in relation to the presence of lymph node metastasis were in the case of E-cadherin at the advancing edge.

In this study, the intratumoral E-caderin-/CD44- immunophenotype, respectively E-caderin-/CD44+ profiles at the advancing edge were associated with the tumor aggressiveness analyzed parameters. It is considered that the existence of a strong CD44 expression in the tumor advancing edge may be an expression of the protein interaction with extracellular matrix in the sense of growth and modulation of tumor progression [22, 24]. Tumor development involves both CD44 connections with extracellular hyalunorate, and signals to other stromal elements (fibroblasts, lymphocytes, angioblasts) [28, 39]. This may explain the multitude of CD44-positive stromal elements near the tumor invasion front. These considerations, coroborated with an increased Ki-67 PI, indicate the existence in the advancing edge of a selection of tumoral cells that became functionally independent from the rest of the tumor, a group of cells with fast multiplication rate and suited to exist in the peritumoral environment, issues identified by some studies that have analyzed the behavior of malignant tumors and EMT process [4, 5, 22, 28, 40]. Nevertheless, the emphasis of EMT process can be difficult because it is possible that only certain cell population in from the advancing edge and only in a limited time window to present such properties [41].

Conclusions

The study indicated the significant decrease of E-cadherin and CD44 expression in aggressive urothelial carcinomas. In addition, high-grade urothelial carcinomas in advanced stage of the disease, with an increased Ki-67 PI, as well as metastasizing carcinomas were characterized by E-cadherin-/CD44+ immunophenotype at the advancing edge. The maintenance of CD44 expression at advancing edge is a negative prognostic factor for bladder urothelial carcinoma and supports the implication of EMT process, through the existence at this level of a cell population with particular properties. Future studies are needed to complete the analysis of cadherins system and their interaction with the intra- and extracellular environment, data that can provide useful information about EMT process, for assessing lesions' prognosis and decide the therapeutic attitude.

Conflict of interests

The authors declare that they have no conflict of interests.

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Author contribution

All authors equally contributed to this paper.

References

[1] Pashos CL, Botteman MF, Laskin BL, Redaelli A. Bladder cancer: epidemiology, diagnosis, and management. Cancer Pract, 2002, 10(6):311–322.
[2] Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer, 2010, 46(4):765–781.
[3] Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol, 2013, 63(2):234–241.
[4] Voulgari A, Pintzas A. Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. Biochim Biophys Acta, 2009, 1796(2):75–90.
[5] McConkey DJ, Choi W, Marquis L, Martin F, Williams MB, Shah J, Svatek R, Das A, Adam L, Kamat A, Siefker-Radtke A, Dinney C. Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. Cancer Metastasis Rev, 2009, 28(3–4):335–344.
[6] Ouyang G, Wang Z, Fang X, Liu J, Yang CJ. Molecular signaling of the epithelial to mesenchymal transition in generating and maintaining cancer stem cells. Cell Mol Life Sci, 2010, 67(15):2605–2618.
[7] Brabletz T. To differentiate or not – routes towards metastasis. Nat Rev Cancer, 2012, 12(6):425–436.
[8] Kiesslich T, Pichler M, Neureiter D. Epigenetic control of epithelial–mesenchymal-transition in human cancer. Mol Clin Oncol, 2013, 1(1):3–11.
[9] Clairotte A, Lascombe I, Fauconnet S, Mauny F, Félix S, Algros MP, Bittard H, Kantelip B. Expression of E-cadherin and alpha-, beta-, gamma-catenins in patients with bladder cancer: identification of gamma-catenin as a new prognostic marker of neoplastic progression in T1 superficial urothelial tumors. Am J Clin Pathol, 2006, 125(1):119–126.
[10] Desai S, Lim SD, Jimenez RE, Chun T, Keane TE, McKenney JK, Zavala-Pompa A, Cohen C, Young RH, Amin MB. Relationship of cytokeratin 20 and CD44 protein expression with WHO/ISUP grade in pTa and pT1 papillary urothelial neoplasia. Mod Pathol, 2000, 13(12):1315–1323.
[11] Lipponen PK, Eskelinen MJ. Reduced expression of E-cadherin is related to invasive disease and frequent recurrence in bladder cancer. J Cancer Res Clin Oncol, 1995, 121(5): 303–308.
[12] Sun W, Herrera GA. E-cadherin expression in invasive urothelial carcinoma. Ann Diagn Pathol, 2004, 8(1):17–22.
[13] Maeda M, Johnson KR, Wheelock MJ. Cadherin switching: essential for behavioral but not morphological changes during an epithelium-to-mesenchyme transition. J Cell Sci, 2005, 118(Pt 5):873–887.
[14] Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. Cell, 1990, 61(7):1303–1313.
[15] Cho SH, Park YS, Kim HJ, Kim CH, Lim SW, Huh JW, Lee JH, Kim HR. CD44 enhances the epithelial–mesenchymal transition in association with colon cancer invasion. Int J Oncol, 2012, 41(1):211–218.
[16] Hu X, Ruan Y, Cheng F, Yu W, Zhang X, Larré S. p130Cas, E-cadherin and β-catenin in human transitional cell carcinoma of the bladder: expression and clinicopathological significance. Int J Urol, 2011, 18(9):630–637.
[17] Shi B, Laudon V, Yu S, Dong D, Zhu Y, Xu Z. E-cadherin tissue expression and urinary soluble forms of E-cadherin in

patients with bladder transitional cell carcinoma. Urol Int, 2008, 81(3):320–324.
[18] Erdemir F, Ozcan F, Kilicaslan I, Parlaktas BS, Uluocak N, Gokce O. The relationship between the expression of E-cadherin and tumor recurrence and progression in high-grade stage T1 bladder urothelial carcinoma. Int Urol Nephrol, 2007, 39(4):1031–1037.
[19] Kong QY, Liu J, Chen XY, Wang XW, Sun Y, Li H. Differential expression patterns of hyaluronan receptors CD44 and RHAMM in transitional cell carcinomas of urinary bladder. Oncol Rep, 2003, 10(1):51–55.
[20] Hong RL, Pu YS, Chu JS, Lee WJ, Chen YC, Wu CW. Correlation of expression of CD44 isoforms and E-cadherin with differentiation in human urothelial cell lines and transitional cell carcinoma. Cancer Lett, 1995, 89(1):81–87.
[21] Lindquist D, Ahrlund-Richter A, Tarján M, Tot T, Dalianis T. Intense CD44 expression is a negative prognostic factor in tonsillar and base of tongue cancer. Anticancer Res, 2012, 32(1):153–161.
[22] Le Bras GF, Allison GL, Richards NF, Ansari SS, Washington MK, Andl CD. CD44 upregulation in E-cadherin-negative esophageal cancers results in cell invasion. PLoS One, 2011, 6(11):e27063.
[23] Liu J, Brown RE. Immunohistochemical detection of epithelial–mesenchymal transition associated with stemness phenotype in anaplastic thyroid carcinoma. Int J Clin Exp Pathol, 2010, 3(8):755–762.
[24] Li W, Liu F, Lei T, Xu X, Liu B, Cui L, Wei J, Guo X, Lang R, Fan Y, Gu F, Tang P, Zhang X, Fu L. The clinicopathological significance of CD44+/CD24-/low and CD24+ tumor cells in invasive micropapillary carcinoma of the breast. Pathol Res Pract, 2010, 206(12):828–834.
[25] Lopez-Beltran A, Sauter G, Knowles MA. Tumours of the urinary system. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA (eds). Pathology and genetics of tumours of the urinary system and male genital organs. World Health Organization classification of tumours, IARC Press, Lyon, 2004, 89–154.
[26] Compérat E, Camparo P, Haus R, Chartier-Kastler E, Bart S, Delcourt A, Houlgatte A, François R, Capron F, Vieillefond A. Immunohistochemical expression of p63, p53 and MIB-1 in urinary bladder carcinoma. A tissue microarray study of 158 cases. Virchows Arch, 2006, 448(3):319–324.
[27] Liao HX, Lee DM, Levesque MC, Haynes BF. N-terminal and central regions of the human CD44 extracellular domain participate in cell surface hyaluronan binding. J Immunol, 1995, 155(8):3938–3945.
[28] Goodison S, Urquidi V, Tarin D. CD44 cell adhesion molecules. Mol Pathol, 1999, 52(4):189–196.
[29] Trochon V, Mabilat C, Bertrand P, Legrand Y, Smadja-Joffe F, Soria C, Delpech B, Lu H. Evidence of involvement of CD44 in endothelial cell proliferation, migration and angiogenesis *in vitro*. Int J Cancer, 1996, 66(5):664–668.
[30] DeGrendele HC, Kosfiszer M, Estess P, Siegelman MH. CD44 activation and associated primary adhesion is inducible *via* T cell receptor stimulation. J Immunol, 1997, 159(6): 2549–2553.
[31] Kim WJ, Park S, Kim YJ. Biomarkers in bladder cancer: present status and perspectives. Biomark Insights, 2007, 2: 95–105.
[32] Xu Y, Yu Q. E-cadherin negatively regulates CD44-hyaluronan interaction and CD44-mediated tumor invasion and branching morphogenesis. J Biol Chem, 2003, 278(10):8661–8668.
[33] Bryan RT, Atherfold PA, Yeo Y, Jones LJ, Harrison RF, Wallace DM, Jankowski JA. Cadherin switching dictates the biology of transitional cell carcinoma of the bladder: *ex vivo* and *in vitro* studies. J Pathol, 2008, 215(2):184–194.
[34] Olivo M, Lucky SS, Kent Mancer JF, Lau WK. Altered expression of cell adhesion molecules leads to differential uptake of hypericin in urothelial cancer. Urol Oncol, 2010, 30(5):624–634.
[35] 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(11): 1194–1205.
[36] Byrne RR, Shariat SF, Brown R, Kattan MW, Morton RA Jr, Wheeler TM, Lerner SP. E-cadherin immunostaining of bladder transitional cell carcinoma, carcinoma *in situ* and lymph node metastases with long-term follow-up. J Urol, 2001, 165(5):1473–1479.
[37] Koksal IT, Ates M, Danisman A, Sezer C, Ciftcioglu A, Karpuzoglu G, Sevuk M. Reduced E-cadherin and alpha-catenin expressions have no prognostic role in bladder carcinoma. Pathol Oncol Res, 2006, 12(1):13–9.
[38] Gontero P, Banisadr S, Frea B, Brausi M. Metastasis markers in bladder cancer: a review of the literature and clinical considerations. Eur Urol, 2004, 46(3):296–311.
[39] Sy MS, Guo YJ, Stamenkovic I. Inhibition of tumor growth *in vivo* with a soluble CD44-immunoglobulin fusion protein. J Exp Med, 1992, 176(2):623–627.
[40] Yildiz E, Gokce G, Kilicarslan H, Ayan S, Goze OF, Gultekin EY. Prognostic value of the expression of Ki-67, CD44 and vascular endothelial growth factor, and microvessel invasion, in renal cell carcinoma. BJU Int, 2004, 93(7):1087–1093.
[41] Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, Baba H, Mori M. Epithelial–mesenchymal transition in cancer development and its clinical significance. Cancer Sci, 2010, 101(2):293–299.

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