Immune expression of E-cadherin and alpha, beta and gamma-Catenin adhesion molecules and prognosis for upper urinary tract urothelial carcinomas

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Immune expression of E-cadherin and α, β and γ-Catenin adhesion molecules and prognosis for upper urinary tract urothelial carcinomas

Sabrina Thalita dos Reis, Katia Ramos Moreira Leite, Alcides Mosconi Neto, José Pontes Júnior, Nayara Izabel Viana, Alberto Azoubel Antunes, Marcos Francisco Dall´Oglio, Miguel Srougi

Laboratory of Medical Investigation (LIM55), Urology Department, University of Sao Paulo Medical School (STR, KRML, AMN, JPJ, NIV, AAA, MS), Sao Paulo, Brazil and Instituto do Câncer do Estado de São Paulo (ICESP), University of Sao Paulo Medical School (JPJ, MFD), Sao Paulo, Brazil

ABSTRACT

Introduction: Cell adhesion molecules (CAM) are required for maintaining a normal epithelial phenotype, and abnormalities in CAM expression have been related to cancer progression, including bladder urothelial carcinomas. There is only one study that correlates E-cadherin and α-, β- and γ-catenin expression with prognosis of upper tract urothelial carcinomas. Our aim is to study the pattern of immune expression of these CAMs in urothelial carcinomas from the renal pelvis and ureter in patients who have been treated surgically. Our goal is to correlate these expression levels and c*haracteris*tics with well-known prognostic parameters for disease-free survival.

Materials and Methods: We evaluated specimens from 20 patients with urothelial carcinomas of the renal pelvis and ureter who were treated with nephroureterectomy or ureterectomy between June 1997 and January 2007. CAM expression was evaluated by immunohistochemistry in a tissue microarray and correlated with histopathological characteristics and patient outcomes after a mean follow-up of 55 months.

Results: We observed a relationship between E-cadherin expression and disease recurrence. Disease recurrence occurred in 87.5% of patients with strong E-cadherin expression. Only 50.0% of patients with moderate expression and 0% of patients with weak or no expression of E-cadherin had disease recurrence (p = 0.014). There was also a difference in disease-free survival. Patients with strong E-cadherin expression had a mean disease-free survival rate of 49.1 months, compared to 83.9 months for patients with moderate expression (p = 0.011). Additionally, an absence of α-catenin expression was associated with tumors that were larger than 3 cm (p = 0.003).

Conclusions: We demonstrated for the first time that immune expression of E-cadherin is related to tumor recurrence and disease-free survival rates, and the absence of α-catenin expression is related to tumor size in upper tract urothelial carcinomas.

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INTRODUCTION

Upper tract urothelial carcinomas (UUC) are defined as tumors that occur in the renal pelvis and ureter, and these carcinomas represent 5 to 8% of all patients with urothelial cancers (1). Additionally, these carcinomas have been associated with aggressive behavior. Previous studies

have suggested that the thin sub-epithelial connective tissue and muscularis propria of the upper urinary tract are responsible for early tumor invasion. Furthermore, diagnosis of this carcinoma tends to be delayed because of a lack of symptoms and a lack of neoplastic cells in urine cytology (2,3).

There are few predictors of tumor recurrence after nephroureterectomy. The main prognostic factors are the patient's age, tumor location, and tumor architecture, stage and histological grade. However, there is currently no evidence that specific tumor characteristics would be able to define patients that could benefit from adjuvant treatment (4).

Biomarkers have not been extensively studied in UUCs. P53 mutations (5) and angiogenesis (6), which are well-known markers of worse prognosis in patients with bladder UCs, have also been related to worse prognosis in patients with UUCs.

E-cadherin is a transmembrane glycoprotein that mediates the selective adhesion of epithelial cells. This protein is required for interactions and maintenance of tissue integrity (7). α-Catenin mediates interactions between the E-cadherin/β-catenin complex and the actin cytoskeleton (8), whereas γ-catenin probably has a role on desmosomal plaques (9).

The role of adhesion molecules in UC is still controversial, and only a few studies have been published in the literature on this topic (6,10). Dysfunctions in cell-cell adhesion molecules have been related to the initial steps of tumor invasion and the development of metastasis in urothelial bladder carcinomas. Recently, Kashibuchi et al. (11) demonstrated aberrant expression of E-cadherin and α-, β- and γ-catenin in 40 to 50% of urothelial bladder carcinoma cases. They also related aberrant expression to tumor stage and cancer specific survival.

The aim of our study is to evaluate the expression of the E-cadherin and α-, β- and γ-catenin adhesion molecules in UUC cells by immunohistochemistry using a tissue microarray construction (TMA) of specimens from 20 patients who underwent ureterectomy or nephroureterectomy for treatment of their UUCs.

MATERIALS AND METHODS

Patients

We retrospectively evaluated surgical specimens from 20 patients with UUC who underwent nephroureterectomy or ureterectomy performed by a single surgeon (MS) from 1997 to 2007. The median age of the patients was 67 years old. Twelve patients had tumors of the renal pelvis, five had tumors of the ureter, and three had multifocal tumors that were present in both the renal pelvis and the ureter. Clinical and pathological characteristics of the patients are listed in Table-1.

All subjects provided informed consent before participating in the study, and the study was approved by the Institutional Board of Ethics (#0720/09).

The specimens were fixed in buffered 10% formalin, routinely processed, stained with hematoxylin and eosin, and examined by a single pathologist (KRML). The prognostic factors that were evaluated included tumor grade, which was determined according to the 2004 WHO classification tumor stage (TNM 2010), tumor size, multicentricity and neoplastic microvascular invasion. The pattern and intensity of immunoexpression of the adhesion molecules were compared to the above prognostic factors and with the disease-free survival rates after a mean follow-up of 55 months.

TMA construction

TMA construction was performed as previously described (12). Slides that contained each of the patients' tumors were selected by examining areas that best represented the entire tumor. Two areas from each tumor were marked with permanent ink to correspond to areas included in TMA analysis. A 1.0 mm punch was used.

To create the slides, a three-micrometer section from the paraffin block was placed onto an adhesive-coated slide. During the heat antigen retrieval process, slides were placed in a citrate buffer (1 mM, pH 6.0) and heated for 30 min in a steamer. Then, slides were incubated overnight at 4°C with a 1:50 dilution of monoclonal antibodies against E-cadherin (Dako Cytomation, CA, USA) and β-catenin (BD San Jose, CA, USA). A

Table 1 - Patients Characteristics.

	Patients(n = 20)
Age (years)	
Mean	66,5
Min - Max	52-85
Gender	
Female	4 (20%)
Male	16 (80%)
Pathological Stage	
pTa-pT1	7 (35%)
pT2-pT3	13 (65%)
Tumor size	
≤ 3 cm	6 (30%)
>3 cm	14 (70%)
Tumor multicentricity	
Yes	5 (25%)
No	15 (75%)
Tumor Grade	
High	15 (75%)
Low	5 (25%)
Microvascular Invasion	
Yes	10 (50%)
No	10 (50%)
Recurrence	
Yes	10 (50%)
No	10 (50%)

1:100 dilution of antibodies against γ-catenin (Zymed Montrouge, France) and α-catenin (Santa Cruz, St Cruz, CA, USA) was used. The LSAB system was used for immunostaining (LSAB; Dako Cytomation, CA, USA). Color was developed by reactions with a 3.3'-diaminobenzidine substrate-chromogen solution followed by counterstaining with Harris hematoxylin. Finally, slides were dehydrated, coverslipped, and examined under light microscope.

The expression of each marker was evaluated by a single pathologist (KRML). Semi-quantitative analysis was performed for all antibodies according to the intensity of staining: negative, weak, moderate and strong (Figure-1).

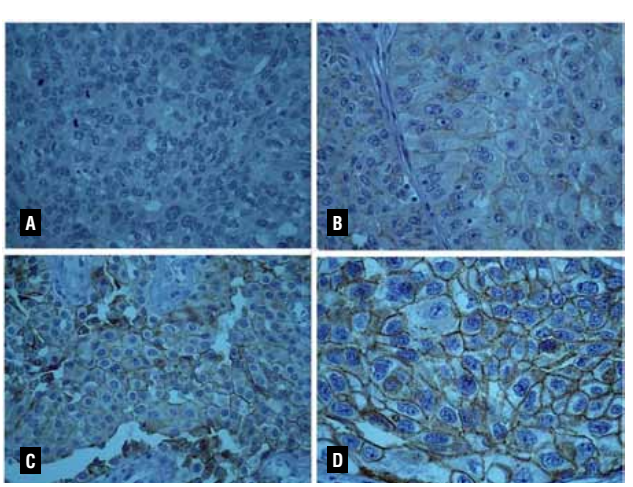
Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for Windows (release 16.02). For comparison between categories, we used an ANOVA and Student's t-tests and significance was determined at $p \leq 0.05$. For adhesion molecule expression profiles with significant associations with recurrence risks, Kaplan-Meier survival curves were used to illustrate disease-free survival. We performed a log-rank test to show differences between curves.

RESULTS

All cases were usual urothelial carcinomas without any other specification. Fifteen (75%) cases were high grade urothelial carcinomas. Only two (10%) cases were staged pTa,

Figure 1 - Examples of the subjective analysis of the intensity of immune expression that was considered as negative (A), weak (B), moderate (C) and strong (D).



five (25%) were pT1, three (15%) pT2 and the remaining 10 (50%) were staged pT3.

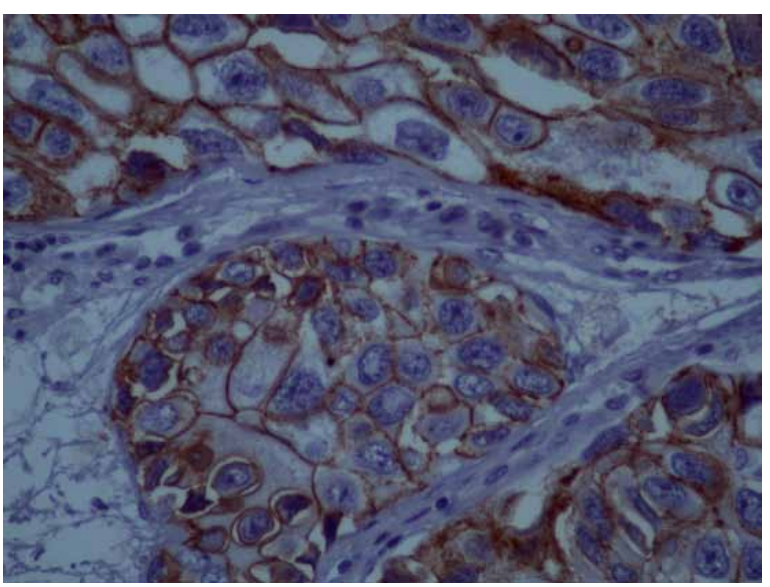
The pattern of staining was membrane for E-chaderin and cytoplasmatic for the other markers. Moderate and strong E-cadherin expression was significantly related to tumor recurrence (p = 0.014) (Figure-2), while moderate γ-catenin expression was correlated with a lower disease-free survival rate with marginal statistical significance (p = 0.071)(Table-2). There was no relationship between β-catenin or α-Catenin expression with tumor recurrence, p = 0.999 and p = 0.465 respectively.

When CAM expression was analyzed in relation to the classical prognostic parameters of pathological stage, tumor grade, microvascular invasion, multicentricity and tumor size, we found that α-catenin expression was negative or weak in 92.9% of tumors that were larger than 3 cm (p = 0.003) (Table-2).

DISCUSSION

When studying immune expression of E-cadherin and α-, β-, γ-catenin in UUC cells, we were able to show that moderate or strong

Figure 2 - Micrography of an IHC reaction showing strong expression of E-cadherin.



Kaplan-Meier curves showed that patients with strong E-cadherin expression had a mean disease-free survival rate of 49.1 months. Patients with moderate expression levels had a mean survival of 83.9 months (p = 0.011) (Figure-3). γ-catenin, β-Catenin and α-Catenin expression levels did not demonstrate differences in disease-free survival length, p = 0.053; p = 0.951; p = 0.913, respectively.

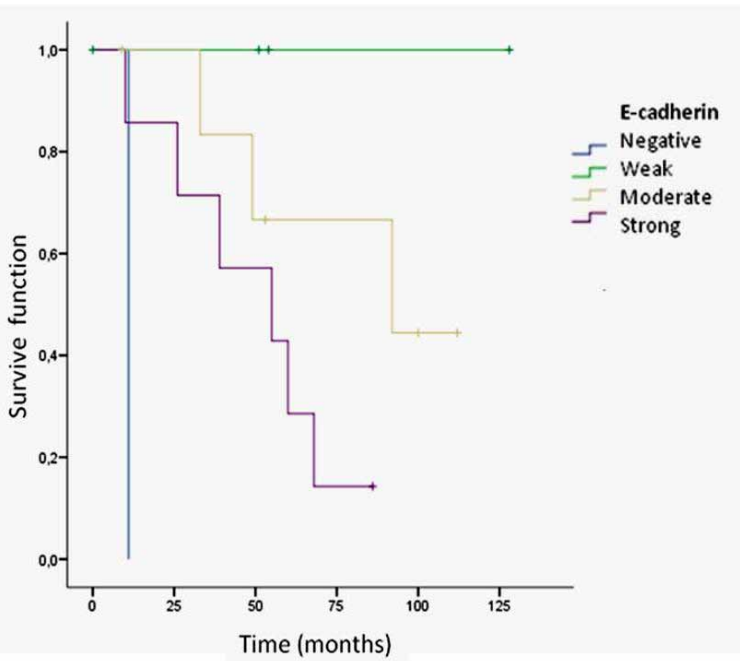
expression levels of E-cadherin were related with both tumor recurrence (p = 0.014) and a shorter time before tumor recurrence (p = 0.011). Additionally, negative or weak expression of α-catenin were correlated with larger tumors (p = 0.003). This is the first study reporting on the relationship between immune expression of members of the cadherin-catenin complex and UUC prognosis.

Table 2 - Correlation between immune expression of E-chaderin, γ-catenin and α-catenin and prognostic parameters and tumor recurrence in 20 patients with UUC.

E–chaderin	Gender		Stage			Grade		Microvascular invasion		Multicentricity		Tumor size		Tumor recurrence	
	F	M	pTa-1	pT2	pT3	Low	High	Absent	Present	No	Yes	≤ 3 cm	> 3 cm	No	Yes
Negative	0	1	0	0	1	0	1	0	1	0	1	0	1	0	1
Weak	1	3	1	1	2	1	3	2	2	3	1	2	2	4	0
Moderate	1	7	3	0	5	1	7	5	3	6	2	1	7	4	4
Strong	2	5	1	2	4	3	4	3	4	6	1	3	4	1	6
P value	0.774		0.626			0.493		0.565		0.354		0.348		0.014	
γ-catenin															
Weak	1	4	1	1	3	1	4	2	3	3	2	0	5	1	4
Moderate	2	8	2	1	7	1	9	4	6	7	3	4	6	7	3
Strong	1	4	2	1	2	3	2	4	1	5	0	2	3	1	4
P value	> 0.999		0.612			0.119		0.282		0.170		0.120		0.071	
α-catenin															
Negative	1	10	4	1	6	3	8	6	5	9	2	0	11	4	7
Weak	2	3	0	2	3	2	3	3	2	3	2	3	2	2	3
Moderate	0	3	1	0	2	0	3	1	2	3	0	2	1	2	1
Strong	1	0	0	0	1	0	1	0	1	0	1	1	0	1	0
P value	0.086		0.362			0.412		0.569		0.149		0.003		0.465	

(F) female; (M) male

Figure 3 - Kaplan-Meier curve for recurrence-free survival according to E-cadherin expression (p=0.011).



E-cadherin and γ-catenin were moderately or strongly expressed in most patients, but α-catenin was absent or lost in the majority of cases.

Cadherins represent a family of transmembrane glycoproteins that mediate homophile, calcium-dependent, intercellular adhesion. E-cadherin is the major cadherin molecule expressed by epithelial cells. Linkage between E-cadherin and intracellular catenins is necessary for the formation of strong intercellular adhesion. β-catenin and γ-catenin bind directly to the cytoplasmic domain of E-cadherin, and α-catenin links the bound β-catenin to actin in the cytoskeleton (13,14).

Several studies have explored the expression of cadherins and catenins and their role as prognostic factors in urothelial bladder carcinomas. These studies suggested that aberrant expression of α-catenin, β-catenin, E-cadherin and γ-catenin correlates with higher tumor stages and worse patient prognosis (15-17). Kashibuchi et al. showed a correlation between aberrant β-catenin and γ-catenin expression with tumor stage and a correlation between γ-catenin with tumor grade in urothelial bladder carcinomas. Furthermore, they found that abnormal expression of E-cadherin was an independent predictor of disease-specific survival in this neoplasia (11). Different from them, we tried to add details to the pattern of immune expression, stratifying the intensity of staining in weak, moderate and strong patterns. The subjective quantification should be better explored in future studies by evaluating adhesion molecules gene expression profiles using quantitative real time PCR that could be proposed as markers to diagnose or follow patients that harbor upper urinary tract urothelial carcinomas.

In our study, we found an association between overexpression of E-cadherin and tumor recurrence. These data contradict previous studies that have demonstrated that loss of E-cadherin is related to higher tumor stages and worse prognosis behavior in many cancers, including bladder tumors. In studies that specifically examined UUCs, Velickovic et al. showed that, in sporadic urothelial carcinomas and in patients

with Balkan endemic nephropathy, lower E-cadherin expression was correlated with higher tumor stages in both groups (18). However, some authors postulate that E-cadherin expression can be reversibly controlled by methylation (19). This methylation would be responsible for E-cadherin down-regulation during the epithelial-mesenchymal transition, which is an important stage in tumor invasion and dissemination (20). But for tumor establishment in the metastatic site there is a requirement for E-cadherin re-expression (21). In our study, we observed re-expression of E-cadherin that was more expressed in pT3 local tumors (69.3%) and that were able to disseminate or recur locally. Interestingly, in agreement with our data, a recent study published by Lim et al. (22) found normal expression of E-cadherin in both usual and micropapillary urothelial bladder carcinomas, and that was independent of tumor stage, tumor grade or presence of microvascular invasion. In their study, they proposed that loss of E-cadherin expression may be a characteristic of a histologically special plasmocytoid or signed ring cell urothelial carcinoma.

Our study also showed that lower expression of α-catenin was associated with tumors larger than 3 cm ($p = 0.003$). Tumor size is a very important prognostic parameter in UC. It is related to tumor recurrence, with 7-fold increase risk in finding tumors after transurethral resection and BCG treatment. Tumor size has also been used as a criteria to proceed with more aggressive treatment in pT1-stage bladder cancer (23,24). Negative expression of this catenin has been related to worse prognosis, tumor stage, tumor grade and UC disease recurrence in other studies (11,25). However, there were no studies of α-catenin expression in UUCs.

Moderate γ-catenin expression was marginally significantly related to tumor recurrence ($p = 0.071$) in our study. The same result was described by Clairotte et al. in cases of bladder cancer. They classified CAM expression as either normal, heterogeneous or absent. The percentage of cases with absence of CAM expression was very low, as was in our series. They showed that heterogeneous expression of γ-catenin was related to worse prognosis and shorter disease-free survival rate in patients with bladder cancer. If our moderate expression category is similar to their heterogeneous category, we observed similar findings in patients with UUCs (25).

Some authors have discussed the accuracy of TMA immunohistochemistry in measuring protein expression (26), but the advantages of the method outperform the disadvantages in terms of standardization of reaction and analysis and have been applied in the evaluation of many prognostic markers in different tumors including urothelial carcinoma for more than 20 years (27).

We are aware that we have studied a small number of cases; however, UUCs are rare and it took a period of 10 years to collect the cases that were included in this study. A larger number of cases would allow analysis of different aspects, such as tumor location. Tumor location is important because it is well known that UCs of the renal pelvis and ureter have different behaviors. Additionally, it is difficult to compare different studies because each study establishes diverse criteria to evaluate immune expression of CAMs. A more standardized results evaluation would make it easier to discover the real role of CAMs in UCs.

However, this is the first study to explore immune expression of components of the cadherin-catenin complex and their role in UUCs.

CONFLICT OF INTEREST

None declared.

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Correspondence address:
Dr. Katia Ramos Moreira Leite
University of Sao Paulo Medical School
Laboratory of Medical Investigation,
Urology Department – LIM55
Av. Dr. Arnaldo 455, 2° andar, sala 2145
Sao Paulo, SP, 01246-903, Brazil
E-mail: katiaramos@uol.com.br