Int Urol Nephrol (2007) 39:1031–1037
DOI 10.1007/s11255-006-9159-5

ORIGINAL PAPER

The relationship between the expression of E-cadherin and tumor recurrence and progression in high-grade stage T1 bladder urothelial carcinoma

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Received: 2 May 2006 / Accepted: 21 November 2006 / Published online: 6 March 2007
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Abstract
Objective To evaluate the relationship between the expression of E-cadherin (E-CD) and tumor recurrence and progression in patients with high-grade stage T1 urothelial carcinoma of bladder.
Methods Fifty-two patients who had primary high-grade stage T1 urothelial carcinoma were enrolled to the study. The pathologic specimens of patients were evaluated and staged as T1a and T1b according to muscularis mucosae involvement by the tumor. The immunohistochemical demonstration of E-CD was accomplished by using immunoperoxidase method and all the specimens were examined under light microscope for E-CD level.
Results The mean age of the patients was 64.0 ± 7.7 (range 36–81) years. The mean follow-up period was 56.4 ± 19.4 (range 14–84) months. Among 52 patients, 27 (52%) of them were stage T1b and 25 (48%) were T1a tumors. The recurrence rates for T1a and T1b groups were 52% ($n = 13$) and 92.6% ($n = 25$), respectively ($P < 0.05$). The expression of E-CD was homogenous in 52% of pT1a and 14.8% of T1b tumors ($P < 0.05$). In T1a group with recurrence, homogeneous E-CD staining ratio was 30.7% ($n = 4/13$), but it was 75% ($n = 9/12$) in T1a patients without recurrence ($P < 0.05$). In T1b group with recurrence, the homogenous expression of E-CD was 12% ($n = 3/25$) and the expression of E-CD was heterogenous in 88% ($n = 22/25$) of them ($P < 0.05$). In T1a group, progression of the disease was detected in 28% ($n = 7/25$) of the patients, but disease progression was seen in 55.5% ($n = 15/27$) of T1b group patients ($P < 0.05$). In T1a group with progression, heterogeneous E-CD staining ratio was 85.7% ($n = 6/7$), but it was 80% ($n = 12/15$) in T1b patients with progression. The effects of tumor number, tumor size and carcinoma in situ presence on recurrence were evaluated within each group. It was determined that parameters such as tumor number and tumor size had no significant effect on recurrence of the groups. The mean survival rates were statistically different between the groups. On multivariate analysis only E-cadherin expression ($P = 0.012$, odds ratio 6.291, 95% confidence

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interval for odds ratio 1.303–4.72) and tumor stage ($P = 0.003$, odds ratio 11.58, 95% confidence interval for odds ratio 2.446–8.542) remained independently significant as predictors of recurrence.

Conclusion E-CD expression was decreased in pathologic specimens of bladder tumor patients with muscularis mucosae involvement and this condition correlated well with tumor recurrence.

Keywords E-cadherin · Bladder · Superficial · Urothelial carcinoma · Progression

Introduction

Urothelial bladder carcinoma is the fourth leading malignancy in men and the seventh cause of cancer-related deaths. Approximately 75% of all bladder cancers are superficial at presentation and most of these superficial tumors can be resected transurethrally [1]. The high recurrence rate is the major problem after local resection of the tumor and also 10–30% of superficial bladder tumors will progress to muscle invasive disease [2, 3]. Although Ta tumors have likelihood of recurrence and display less propensity to progress, T1 tumors have a greater rate of progression, reaching 30% and even 50% in some instances. Many parameters have been evaluated to find prognostic factors for patients with T1 bladder tumors. Currently, there is no specific tumor marker, which is routinely used for detection and clinical management of urothelial carcinoma (UC) of bladder. Although tumor grade and pathological stage have been the most important factors for predicting the prognosis of bladder cancer, in recent years a variety of tumor markers and specific tests have been used to predict the progression of UC [4–6]. Cadherins are a family of transmembrane glycoproteins that are involved in homotypic calcium-dependent intercellular adhesions [5]. E-Cadherin plays an important role in establishment and maintenance of tissue architecture [6].

In this study, we researched the relationship between E-CD expression and tumor recurrence and progression in patients with high-grade stage T1 urothelial carcinoma of bladder.

Materials and methods

Between January 1997 and December 2006, transurethrally resected bladder tumor specimens of patients with primary high-grade stage T1 urothelial carcinoma of bladder were evaluated. After a complete physical examination all of the patients were evaluated with urinary ultrasonography, intravenous urography and computerized abdominal tomography if indicated. For T1 staging, 2002 TNM system was used [7]. Staging for depth of lamina propria involvement was assessed using the criteria described by Angulo et al. [8]. So, the pathologic specimens of patients were evaluated and staged as T1a (Group 1) and T1b (Group 2) according to muscularis mucosae involvement by the tumor. The data about microstaging distribution, tumor size, multiplicity, presence of carcinoma in situ and survival rate of tumors between these two groups were compared. None of the patients had received prior chemotherapy or radiation therapy. Patients with sarcomatoid diferentiation and papilloma of bladder were excluded from the study. The surgical specimens were graded histologically as high or low grade according to the 2004 Consensus Group grading system [9]. After immunohistochemical staining the specimens were examined under high magnification light microscope for E-CD level.

All immunohistochemical analyses were performed in routinely processed formalin-fixed, paraffin-embedded tissue sections in 3–5 μm-thickness and demonstration of E-CD was achieved by using the immunoperoxidase method. The tissue sections were dewaxed, rehydrated and incubated with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. After rinsing with 0.01 M phospate-buffered saline, normal goat serum was applied for 20 min to block non-specific antibody binding. Six milliliters of mouse monoclonal anti-E-CD antibody solution (Zymed, South San Francisco, CA, catalogue number: 871-4494, 08-1223, USA) was used to determine E-CD molecules in the pathologic specimens.

A semiquantitative evaluation of E-CD immunostaining was carried out by two independent observers. E-CD tissue level was determined according to the percentage of positive cells and

staining pattern of the tissue sections. The adjacent normal urothelium was used as an internal positive control thus allowing direct comparison within the same tissue specimen. Tumors were classified as; (a) homogenous E-CD staining, if more than 90% of tumor cells demonstrated a membranous staining localized at the intercellular borders and exhibited a homogenous pattern of immunoreactivity, identical to that observed in normal urothelium (Figs. 1 and 2), (b) heterogenous E-CD staining, if immunoreactivity was seen in less than 90% of tumor cells (Figs. 3 and 4). The relationship between E-cadherin expression and several parameters such as recurrence and progression were compared in patients with T1a and T1b groups.

The comparison of age, between patients with stage T1a and patients with stage T1b was analyzed by the two independent sample *t*-test. Discrete variables were tested by chi-square test and Fisher's exact test, as applicable. Results were presented as mean ± SD for quantitative variables and number of patients (%) for qualitative variables. Multivariate analysis was evaluated according to the forward stepwise logistic regression model. For all tests, $P < 0.05$ was considered to indicate statistically significant difference. All analyses were performed with SPSS® 14.0 (demo) for windows.

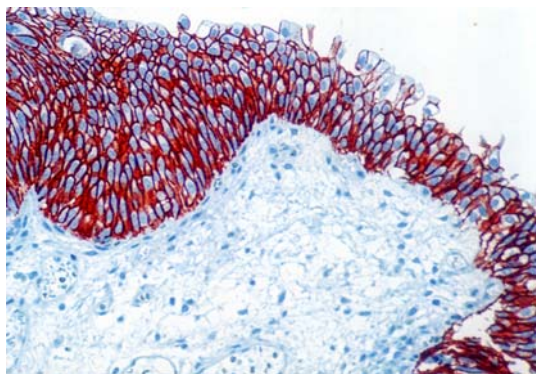


Fig. 1 Diffuse and membranous homogenity in normal mucosae surrounding the tumor (E-CD antibody, ×310)

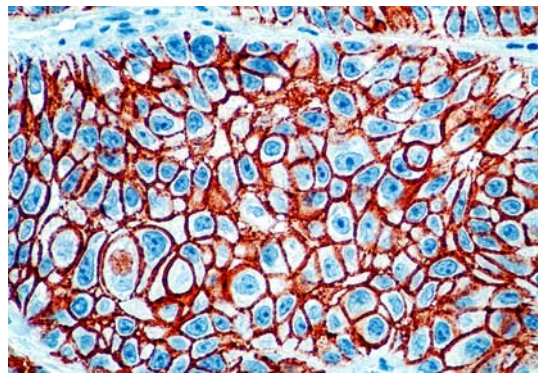


Fig. 2 Diffuse and membranous strong homogenity within the tumoral areas (E-CD antibody, ×500)

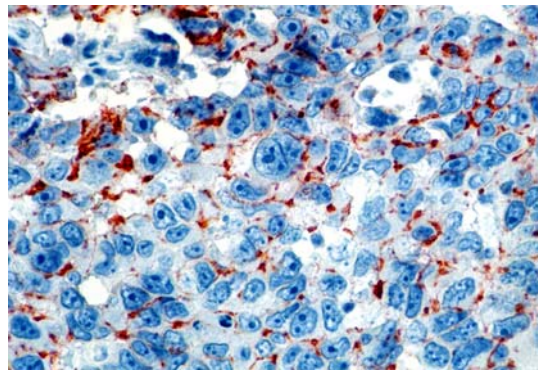


Fig. 3 Focal and weak membranous staining within the tumoral areas (E-CD antibody, ×310)

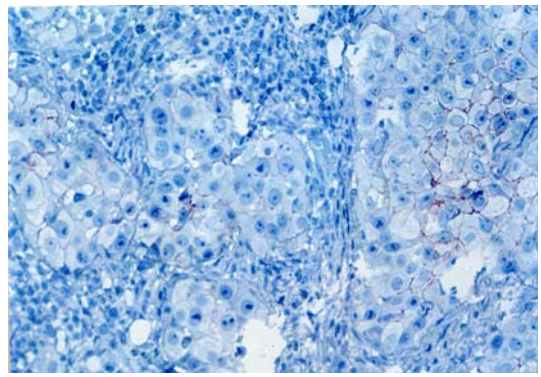


Fig. 4 Focal, weak membranous and intermittant staining of the tumor (E-CD antibody, ×500)

Results

The mean age of the patients was 64.0 ± 7.7 (range 36–81) years and the male to female ratio

Table 1 The rate of E-cadherin expressions for the whole group of patients

Group (n = 52)	Tumor stage		P
	T1a	T1b	
E-cadherin (+) homogenous	13 (52%)	4 (15%)	<0.05
E-cadherin (–) heterogenous	12 (48%)	23 (85%)	
Total	25	27	

Table 4 The rate of E-cadherin expressions in high grade, stage pT1a tumors with or without progression

Group (n = 25)	T1a		P
	Progression (+)	Progression (–)	
E-CD (+) homogenous	1 (4%)	12 (48%)	<0.05
E-CD (–) heterogenous	6 (24%)	6 (24%)	

was 36/16. The mean follow-up period was 56.4 ± 19.4 (range 14–84) months. Among 52 patients, 27 (52%) of them were stage T1b and 25 (48%) were stage T1a bladder UC. Homogenous E-CD staining rates were 52% (13/25) in T1a group and 14.8% (4/27) in T1b group ($P < 0.05$) (Table 1). The recurrence rates for T1a and T1b groups were 52% (13/25) and 92.6% (25/27), respectively. In T1a group with recurrence, homogenous E-CD staining was 30.7% (n = 4/13) whereas in patients with no recurrence this rate was 75% (9/12) ($P < 0.05$) (Table 2). In T1b group with recurrence, homogenous E-CD staining rate was 12% (3/25) and the rate of heterogenous E-CD expresssion was 88% (22/25) ($P < 0.05$) (Table 3). In T1a group, progression was seen in 7 (28%) patients; 1 (4%) with E-CD homogenous and 6 (24%) with heterogenous staining ($P < 0.05$). Progression of the disease was detected in 15 (55.5%) of stage T1b patients, 3 (20%) patient with homogenous and 12 (80%) with heterogenous E-CD expression ($P < 0.05$) (Tables 4 and 5). The effects of tumor number, tumor size and carcinoma in situ presence on recurrence were evaluated within each group. It was determined that parameters such as tumor number and tumor size had no significant effect on recurrence of the groups (Table 6). The mean survival rates in patients with and without E-CD homogeneous were 76.47% and 48.57%, respectively ($P < 0.05$). On multivariate analysis, only the microstaging (T1a and T1b) and E-cadherin expression of urothelial cell carcinoma were

Table 5 The rate of E-cadherin expressions in high grade, stage T1b tumors with or without progression

Group (n = 27)	T1b		P
	Progression (+)	Progression (–)	
E-CD (+) homogenous	3 (11.11%)	1 (3.70%)	<0.05
E-CD (–) heterogenous	12 (44.44%)	11 (40.74%)	

Table 2 The rate of E-cadherin expressions in high grade, stage T1a tumors with or without recurrence

Group (n = 25)	Tumor stage T1a		Total	P
	Recurrence (+)	Recurrence (–)		
E-cadherin (+) homogenous	4 (16%)	9 (36%)	13 (52%)	<0.05
E-cadherin (–) heterogenous	9 (36%)	3 (12%)	12 (48%)	
Total	13 (42%)	12 (48%)	25 (100%)	

Table 3 The rate of E-cadherin expressions in high grade, stage T1b tumors with or without recurrence

Group (n = 27)	Tumor stage T1b		Total	P
	Recurrence (+)	Recurrence (–)		
E-cadherin (+) homogenous	3 (11.1%)	1 (3.7%)	4 (14.81%)	<0.05
E-cadherin (–) heterogenous	22 (81.4%)	1 (3.7%)	23 (85.1%)	
Total	25 (92.6%)	2 (7.4%)	27 (100%)	

Table 6 The effect of tumor size, number and presence of carcinoma in situ on recurrence

Groups	Recurrence (+)	Recurrence (–)	*P*
Tumor number			
T1a (n = 25)			χ^2 = 0.031,
Solitary	2	3	P = 0.859
Multiple	10	10	
T1b (n = 27)			
Solitary	6	1	
Multiple	19	1	
Carcinoma in situ (n = 7)			
T1a	3	–	P = 0.545*
T1b	4	–	
Tumor size (cm)			
T1a	2.8 (0.6–5.6)	2.9 (0.7–5.6)	t = 0.634,
T1b	3.1 (0.9–7)	3.2 (0.9–7)	P = 0.529

* Fischer's exact chi-square test

observed to be independent prognostic predictors of recurrence (Table 7).

Discussion

Stage T1 urothelial carcinoma of bladder comprises about 30% of all superficial bladder cancers and generally have high-grade properties pathologically [3, 10]. Only 30% of patients with stage T1 bladder cancer remain tumor free for 3 years without adjuvant therapy [10]. Tumor recurrence occurs in about 80% of patients with high-grade tumors and more than a half of these cases progress to invasive disease [11]**.** Controversies about treatment and follow-up of high-grade T1 urothelial carcinoma of bladder are still going on, rendering these tumors having a special place in all bladder malignancies [10–12]. Stage T1 tumors have a greater rate of progression, reaching 30% and even 50% in some instances. The muscularis mucosaee, which is comprised of scattered, muscular fibers running along large blood vessels, has changed T1 bladder staging and led to its classification into three groups and, subsequently, into two groups. Angulo et al. proposed that only two populations should be differentiated [8, 13]. Tumors superficial to the muscularis mucosaee or reaching the muscularis mucosaee but not passing through it were classified as T1a, and tumors passing through the muscularis mucosaee and invading the submucosaee were considered to be T1b. Similar classification was used in our study. Although, a consensus on this issue has not been reached, it has been proven that this subclassification has some extent of value on prognosis. Numerous studies have shown that T1b tumors behave more aggressively and have a greater probability of progressing to muscle invasive disease and result in metastasis when compared to T1a tumors [3, 8, 11, 12]. Besides, it is not always possible to predict in which patients recurrence and progression will occur.

Table 7 Clinical and demographic characteristics of the patients

	Group 1 (n = 25) mean±sd	Group 2 (n = 27) mean±sd	t	p
Age	59,12±11,25	61,15±9,52	0.704	0.485
	Group 1 (n = 95) n (%)	Group 2 (n = 23) n (%)		
E-cadherin				
Homegenous	12 (48,0)	23 (85,2)	χ^2 = 6.55, p = 0.010	
Heterogenous	13 (52,0)	4 (14,8)		
Recurrence	13 (52,0)	25 (92,6)	χ^2= 8,906, p = 0.003	
Progression	7 (28,0)	15 (55,6)	χ^2 = 2,988, p = 0.043	
	E-cadherin (+)	E-cadherin (–)		
Survival rates	76.47%	48.57%	p<0.05	

In addition to tumor grading and pathological staging, the loss of expression of ABO blood group antigens, cell adhesion molecules, growth factors, chromosomal and genetic alterations, tumor antigens, anaeuploid DNA content, overexpression of oncoproteins and cytoceratin have been reported as prognostic factors for bladder cancer [14, 15]. Recently, many authors have investigated E-CD expression in normal and transformed urothelium but neither of these review studies had argued about the expression of cell adhesion molecules as potential prognostic factors in high-grade stage T1a and T1b urothelial carcinoma of bladder [5, 6, 16].

The initial step in the local invasion of malignant epithelial tumors is the detachment of cells

from the original position within the epithelial sheet [6, 17]. This process involves loss of intercellular adhesiveness due to altered morphology of specific junctions in epithelial tumors, but the mechanisms of this process is poorly understood [18]. Recent studies suggest that decreased E-CD immunoreactivity correlates with lack of differentiation and aggressiveness of the tumors for carcinomas of many organs such as breast, stomach, rectum, head and neck, lung, bladder and some gynecologic malignancies [19–22]. Demonstration of a casual relationship between impaired E-CD staining and invasive character of a tumor has also been performed experimentally [23]. In this regard, our study on bladder cancer revealed a potential availability of E-CD expression as a prognostic factor.

Using fresh frozen samples and anti-E-CD monoclonal antibody, Bringuier et al., showed that there was loss of E-CD staining associated with increasing histological grade and pathological stage of the tumor [19]. In our study, we also showed a heterogenous E-CD staining pattern in 85.1% (23 of 27) of stage T1b tumors. But in T1a tumors the ratio of E-CD staining loss was 48% (12 of 25) and there was a statistical difference between these values. The strong correlation with tumor stage and grade seems to indicate that disturbance of E-CD expression indeed plays a major role in invasive behavior of bladder tumors. Nakopoulou et al. [24] reported similar findings.

In our study, the expression of E-CD was decreased in T1b group compared to T1a group and it correlated well with tumor recurrence. These results about the association of E-CD expression with tumor recurrence were in agreement with findings of Otto et al. [25] likewise in another study Sun and Herrera [26] reported that the loss of E-CD staining was in relation with tumor recurrence. It is also well known issue that recurrence rates in stage T1b tumors are higher than stage T1a tumors. Herein, it may be thought that E-CD expression does not add additional information for the literature with regard to recurrence rate. However, the most important new knowledge in this study is that; in T1a and T1b groups, E-CD expression was different among patients with and without tumor recurrence. This significantly positive correlation with tumor recurrence also indicates that, altered E-CD expression plays an important role in aggressive behavior of bladder tumors. Similar to the results of previous studies, we found a correlation between E-CD expression and progression of tumor in patients with high-grade stage T1 UC [26]. According to Byrne et al. [27], there is an association between E-CD expression and survival in bladder cancer patients. We have also realized similar results.

It has long been known that lamina propria invasion, carcinoma in situ, tumor number, high grade and tumor size of presenting tumor are associated with recurrence and progression [26]. However, these classic prognostic factors do not provide accurate guidelines for timely treatment in all patients [3, 28]. In present study, several traditional prognostic factors appeared to be of limited value in predicting recurrence and progression in univariate and multivariate analysis. In the studies about recurrence and progression of T1 tumors, which divides the patients into subgroups, it has been observed that recurrence and progression of high- and low-grade tumors had been evaluated together. In present study, we only researched isolated high-grade T1 tumors. In our study, this situation may explain that why classical prognostic factors had no statistically significant effect on progression and recurrence. Similarly, Smits et al. [28] and Sozen et al. [3] had reported that they had not found significant difference between T1a and T1b groups with regards to tumor size, tumor multiplicity and grade.

Conclusion

We have compared the prognostic value of E-CD staining for recurrence and progression in high-grade stage T1 UC of bladder. Although, this retrospective study had been performed in a limited number of cases, to our knowledge, this is the first report, which shows the correlation between E-CD expression and high-grade stage T1 UC of bladder. In addition, E-CD heterogenity was found more frequently in patients with

stage T1b tumor. For this reason we can say that E-CD expression may be considered as a useful prognostic factor in patients with high-grade stage T1 urothelial carcinoma of the bladder.

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