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ARTICLE

E-Cadherin Expression in Transitional Cell Carcinomas

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The authors analyzed the expression of E-cadherin, one of the most important cell adhesion molecules, on paraffin sections of tumors of bladder cancer patients. The aim of the study was to see whether there is any association between E-cadherin expression and tumor grade, stage, age and gender of the patients, number of recurrences, or overall survival. The samples were examined in 51 primary bladder transitional cell carcinomas (TCC) of 50 patients, resected by transurethral resection (TUR) between January 1, 1996 and January 1, 1997. Immunoreactions were performed with monoclonal anti-human E-cadherin antibody. Forty of the fifty patients could be clinically followed. The analysis of the results on these forty patients was performed by contingency analysis and significance was assessed by χ^2 test. No significant association between E-cadherin expression and tumor grade, stage, age or gender of the patients, the number of recurrences, or overall survival could be seen. (Pathology Oncology Research Vol 12, No 2, 73–77)

Key words: bladder cancer, E-cadherin, transitional cell carcinoma

Introduction

E-cadherin is one of the most frequently examined molecules, which has a cardinal role in cell adhesion. The role of adhesion molecules is multiple, since changes in their expression influence the motility of tumor cells, and can facilitate the occurrence of metastasis.

E-cadherin is coded by the CDH1 gene, which is found on locus 22.1 of the long arm of chromosome 16 (16q22.1). The loss of its expression can be due to deletion, point mutation, or the hypermethylation of its promoter.[5] The significance of the latter in TCC has been examined by Horikawa et al. They found an association between hypermethylation of the promoter of gene CDH1, and abnormal E-cadherin expression.[9]

In the United States, bladder cancer is the fifth most frequent solid malignant tumor, with 54,000 new cases diagnosed every year, causing 12,000 deaths.[14] Bladder cancer is the second most frequent urological malignancy. Histologically, 94% of these tumors are transitional cell carcinomas. The leading symptom is macroscopic or microscopic hematuria. Diagnosis is given after histological examination of the biopsy specimen. Further treatment depends on the histological grade and stage of the tumor.[22,24]

There are two main groups, superficial (Ta, T1), and highly invasive (T2-4) tumors. The superficial tumors are further divided into low- (G1) or high-risk (G2,3, CIS) cases.

Beside the histological grade of the tumor, clinical stage also has importance. Generally more superficial tumors are better differentiated histologically (showing a lower grade), and more invasive ones are less differentiated (G3, or anaplastic). Five to 30% of superficial tumors become invasive and/or give metastasis. The aim of many studies is to find those factors that can predict the potential biological behavior of cancers.[1,7,8,15,20,23,25] If the more aggressive behavior of certain tumors could be estimated, the patients having higher risk tumors could be followed more frequently, or treated more radically.[24]

The aim of our study was to determine E-cadherin expression in bladder cancer biopsies and its possible associations with tumor grade, stage, age and gender of the patient, recurrence rate and overall survival.

Materials and Methods

We examined the intensity of E-cadherin expression in tissue specimens obtained by TUR from fifty primary bladder cancer patients. The biopsies were taken between Janu-

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ary 1, 1996, and January 1, 1997. Hematoxylin-eosin staining and immunohistochemical reactions were performed on slides from formalin-fixed, paraffin-embedded material. We used monoclonal anti-human E-cadherin antibody (NLH 38, DakoCytomation, Glostrup, Denmark; dilution 1:120). The intensity of the reactions was assessed semiquantitatively in four categories: - (negative), +/- (uncertain positivity), + (weak reaction), ++ (as strong positivity as in normal urothelium) (*Figure 1*). Grade and stage of the tumors were reassessed. The results were examined by contingency analysis, and significance was assessed with χ^2 test.

The recurrence rate of the tumors, the possibility of recurrence, and overall survival were reassessed in 2004, eight years after the initial biopsy. Overall survival, evaluated in 2004, was divided into three categories: 1: shorter than 5 years, 2: longer than 5, but shorter than 8 years, 3: longer than 8 years. Ten of the fifty patients were lost for follow-up, so the examination could be performed in forty

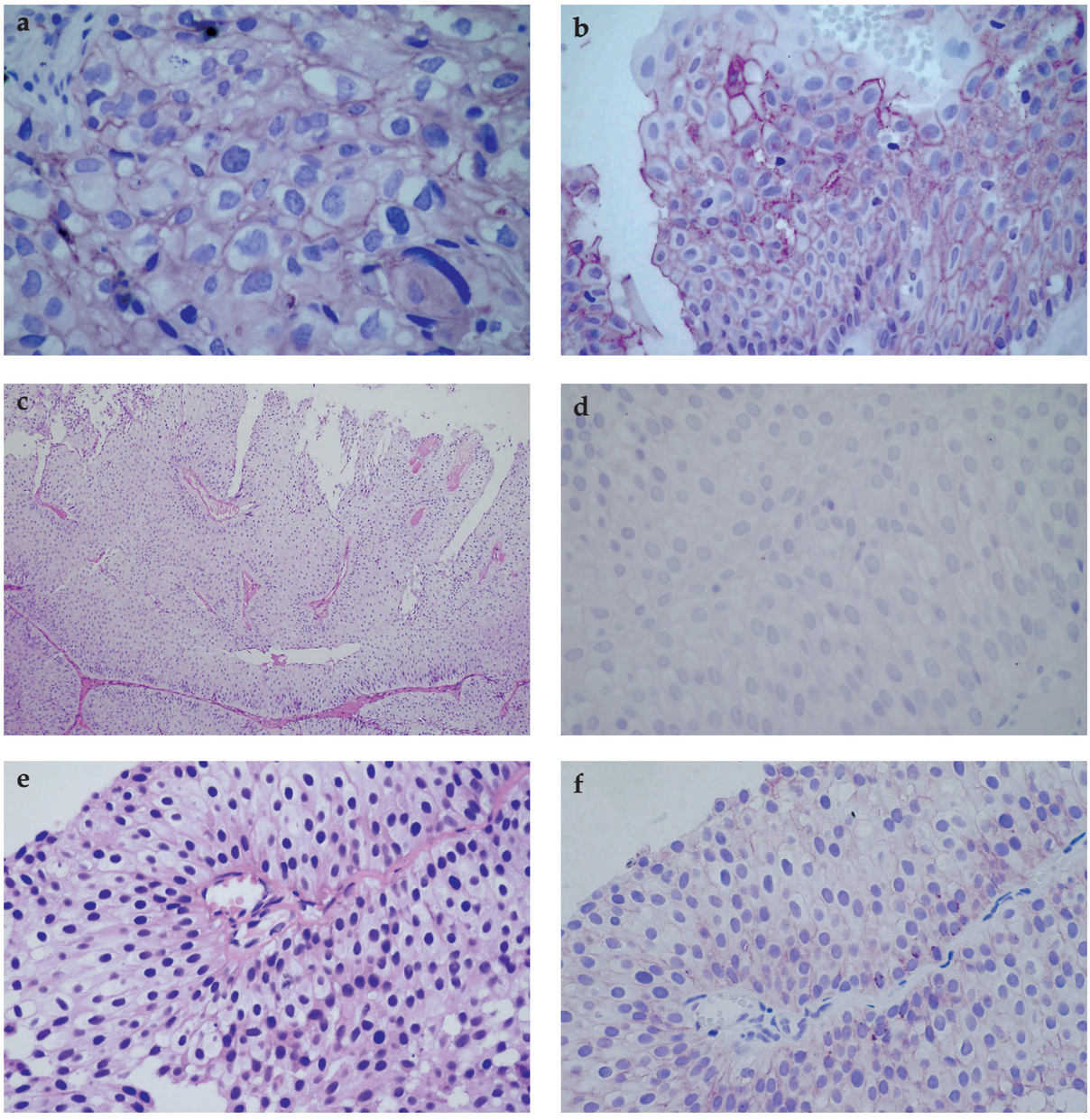

Figure 1. *Morphology and E-cadherin expression in transitional cell carcinoma.* ***a).*** *Grade 3 tumor, E-cadherin positivity assessed as ++.* ***b)*** *Grade 2 tumor, E-cadherin positivity assessed as ++.* ***c)*** *Grade 1 tumor, hematoxylin-eosin.* ***d)*** *Grade 1 tumor, E-cadherin expression assessed as negative.* ***e)*** *Grade 1 tumor, hematoxylin-eosin.* ***f)*** *Grade 1 tumor, E-cadherin positivity assessed as +.*

cases. From the point of recurrence rate, four categories had been established: A: recurrence in the first two years, B: recurrence between 2-4 years, C: recurrence after four years, D: no recurrence (*Table 1*).

Results

Forty of the 50 bladder cancer patients regularly appeared on control examinations, while ten patients were lost for further controls. E-cadherin expression was absent in 13/40 (32%) of the patients. In 25/40 (62%) cases E-cadherin expression was retained, either as strong as in normal epithelium (++), or retained but weak (+). Expression was dubious in 2 further cases. There was no association between E-cadherin expression and tumor grade or stage, age or gender of the patient. Two cases, initially diagnosed as invasive carcinomas appeared to be inverted papillomas on review of the slides. Neither of them showed normal E-cadherin expression, it was retained but week (+) in both.

Table 1. **Age and gender of the patients, grade and stage of the tumors, the intensity of E-cadherin expression, and recurrence and survival categories.**

Patient no.	*Grade*	*Gender*	*TNM*	*E-cad.*	*Survival cat.*	*Recurrence cat.*	*Age of patient in 1996*
1	I	M	Tx	+	3	d	59
2	I	F	Ta	-	3	b	50
3	I	M	Ta	+	1	d	75
4	I	M	Tx	+	3	d	66
5	II	F	T1	+	2	b	73
7	III	F	Ta	++	3	b	59
8	I	M	Ta	+	3	d	54
10	II	F	T1	-	3	d	82
11	II	F	Tx	++	3	d	71
12	I	F	Ta	+	3	d	69
13	I	F	Tx	-	3	b	71
14	I	M	Ta	+	3	d	50
15	I	M	Tx	+	1	d	73
18	II	M	T1	+	1	d	81
19	I	M	Ta	-	1	d	87
20*	IP	M	Tx	+	3	d	29
21	II	M	T1	+	1	a	57
22	II	F	T1	++	2	a	86
25	I	M	Ta	-	3	d	69
27	III	F	T1	++	1	d	70
28	II	M	T1	-	3	d	75
29	I	M	Ta	-	3	d	55
30*	IP	M	Ta	+	1	d	49
31	I	M	Tx	-	1	b	76
32	I	F	Ta	+	1	d	63
33	I	M	T1	-	1	b	50
34	I	F	Ta	-	2	d	76
35	I	M	T1	-	3	d	72
36	II	M	T1	+	3	a	70
38	II	M	T1	-	3	d	40
39	I	M	Ta	-	2	d	75
40	I	M	Ta	+/-	3	d	51
41	I	M	Ta	+	3	d	40
43	II	M	Ta	+	1	b	66
44	III	M	T1	+	2	d	67
45	III	F	Cis	+	3	c	57
46	III	F	Cis	+/-	3	d	50
49	I	F	Ta	+	3	d	
50	II	M	Ta	++	1	d	64
51	III	F	Ta	+	1	d	71

IP: inverted papilloma, Cis: carcinoma in situ

Recurrence rate was also examined in 40 of the patients. Eleven patients had recurrence (categories A, B, C), but no association could be found with E-cadherin expression, using contingency analysis.

Eighteen patients had died within eight years after TUR, 13 of them within five years. In 13 of these cases (13/18, 72%), E-cadherin expression in the primary tumor was either ++ or +, and only 5 (5/18, 28%) showed absence of expression. Similarly to other patient or tumor parameters, overall survival did not show association with the intensity of E-cadherin expression.

Discussion

E-cadherin is a Ca^{2+}-dependent cell adhesion molecule, which belongs to the cadherin family. It is expressed on the surface of epithelial cells, taking part in the zonula adherens. Intracellularly, the molecule forms complexes with catenins (β, γ), and α-catenin attaches the complex to actin filaments of the cytoskeleton.[10,19] Beside playing role in the integrity of epithelial cells, it also takes part in neurulation, during fetal development, since its expression is decreased during detachment from the ectoderm.

E-cadherin is mentioned as a tumor suppressor in the literature, though the role of adhesion molecules is controversial. Their presence hampers the detachment of tumor cells from the original epithelial tissue, lowers their motility, however, it can help the occurrence of distant metastasis. The presence of E-cadherin can be pivotal in the differential diagnosis of certain tumors (ductal/lobular breast cancer).[2] The loss of expression is quite frequent in tumors and can play a role in progression. Expression can be retained in certain cases by the administration of vitamin D.[4] However, in different studies there are conflicting results concerning the presence or absence of E-cadherin expression in different human tumors.

Some of these publications prove that the loss of E-cadherin (and/or catenin) expression is associated with poor prognosis, high tumor grade and stage, while others question or deny the existence of such associations.[3,12,13,16,17,21] However, these differences might be explained by the fact that the loss of E-cadherin expression is associated with disturbances at different levels (mRNA or protein), or with disturbances of the expression of the E-cadherin-associated catenins, or, as found by Bringuier et al in some bladder cancer cell lines, the loss of E-cadherin parallels with the up-regulation of N-cadherin, a molecule found in neural tissues.[2]

The immunohistochemical analysis of E-cadherin expression in bladder cancer biopsy specimens may have prognostic significance. According to the present examination, there is no significant association between E-cadherin expression and tumor grade, stage, or overall survival in bladder transitional cell carcinomas. Our results are similar to those of Koksai et al, who recently published a study on tissues of bladder cancer patients. [11] In contrast to several previously described studies,[3,9,13,16,18,21] they also showed that abnormal E-cadherin expression had no association with tumor grade, recurrence rate and overall survival. However, unlike in our study, in their cases abnormal E-cadherin expression was associated with advanced tumor stage.[11] Further studies with higher numbers of patients, and still longer survival data are needed to see the relevance of either above mentioned associations.

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