Expression of E-cadherin in Human Bladder Transitional Cell Carcinoma

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Abstract **Objective** To investigate the clinical significance of E-cadherin (E-CD) and E-CD mRNA expression in the tissues of bladder transitional cell carcinoma (TCC). **Methods** 102 specimens of bladder transitional cell carcinoma and 10 specimens of normal tissues of human bladder were stained immunohistochemically with anti-E-cadherin monoclonal antibody; 24 specimens of bladder TCC and 16 specimens of normal tissue of human bladder mucosa were measured by the means of RT-PCR. All the results were analyzed statistically. **Results** The expressions of E-cadherin in bladder TCC correlated well with grade, stage and recurrence of the tumor, but had no significant correlation with the magnitude and number of the tumors. **Conclusion** Expression of E-cadherin in the tissue of TCC was closely associated with the biological behaviors of bladder TCC, which may aid early detection and prediction of the clinical course of this disease.

Key words Bladder neoplasms; Carcinoma; Transitional cell; E-Cadherin

Carcinoma of the bladder epithelium is the most common tumor of the genitourinary tract, with transitional cell carcinoma accounting for 90% of all bladder malignancies. The high rate of disease recurrence and metastasis in bladder TCC underscores the need for its early detection and treatment. In recent years, the association of E-cadherin with bladder TCC has been proposed [1]. The cadherins (CDs) form transmembranous Ca^{2+}-dependent cell-cell adhesion receptors that play a major role during embryonic development and in the maintenance of adult tissue architecture [2, 3]. Decreased, heterogeneous or absent expression of membrane-associated E-cadherin has been reported in malignant bladder tumors [4]. Loss of E-CD immunoreactivity leads to dissociation of cells from coherent tissues and generates dedifferentiation and invasiveness in the carcinoma, which suggests E-CD functions as an invasion suppressor molecule [5, 6].

MATERIALS AND METHODS

Clinical data

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102 patients with bladder TCC from Qilu Hospital of Shandong University, China, were used in this study, among which 79 were males and 23 were females. These patients were aged between 29 and 85 years old with the average of 59.4 years. The bladder tumors were classified based on stages and grades (WHO). Their clinical stages were determined according to the criteria set by the International Union Against Cancer (UICC, 1992).

Specimens

Bladder TCC tissue samples were taken from 102 patients who underwent operation. Normal bladder mucosa tissues were obtained from 10 patients with benign prostatic hyperplasia (BPH).

Reagents and instruments

The following is a list of reagents and instruments that were used in this study: E-CD monoclonal antibody (CHEMICON incorporation). HISTOGRIP™ reagent, ILI-9013 pepsin digestion fluid, ILI-9036AEC developer, sheep anti-human blockage serum and horseradish enzyme labeling strepto-avidin were purchased from Beijing Zhongshan biotechnological limited company.

Laboratory procedures

The expression of E−CD in tumor and normal bladder tissue was assessed immunohistochemically using mouse monoclonal antibody (McAb) against E−CD (HECD−1, human epithelial cadherin, 1:400) and avidin−biotin−peroxidase complex (ABC) method on de−paraffinized sections. The samples were labeled as having a normal pattern of E−CD immunoreactivity if the staining was similar to that of normal bladder epithelial cells (i.e., more than 90% of cells stained positively on plasma membrane), and having an abnormal pattern if the results were negative (i.e., complete absence of immunoreactivity) or heterogeneous (i.e., less than 90% of tumor cells with positive membranous staining).

Statistical analysis

Statistical analysis was carried out with SPSS−PC (SPSS Inc, Illinois, U.S.A). Data in different groups were analyzed with chi square test and sum of ranks and $P<0.05$ was considered as significant difference.

RESULTS

The expression of E−CD:

There was normal preserved expression in 10 cases of normal tissue of bladder. The rates of preserved expression in human bladder TCC were as the followings: G1: 88.89%, G2: 57.45%, G3: 28.57%, and there were significant differences between them ($P<0.01$); Tis−T1: 81.25%、T2−T4: 47.14%, and there was significant differences between them ($P<0.01$). With regard to first occurring and recurrence, the preserved expression rate were 67.21% and 43.90% respectively, and there was significant difference between them ($P<0.05$); The recurrence rate in cases (71 cases) of preserved expression (40 cases) and loss expression (31 cases) was 22.5% and 54.8%, there was significant difference between them ($P<0.01$); The preserved expression rate in mono−occuring cases and multi−occuring cases was 58.82% and 56.86% respectively, there was not significant difference between them ($P>0.1$); The preserved expression rate in cases with the diameter below 2cm and those above 2cm were 62.16% and 55.38% respectively, there was

Table 1. Relationship between expression of E−CD and bladder TCC

Index		Case(n)	Expression of E−cadherin	
			Normal (%)	Abnormal (%)
TCC Grade	G1	27	24(88.89)	3(11.11)
	G2	47	27(57.45)★	20(42.55)★
	G3	28	8(28.57)★△	20(71.43)★△
TCC Stage	Tis~T1	32	26(81.25)	6(18.75)
	T2~T4	70	33(47.14)★★	37(52.86) ★★
TCC occurrence	Primary	61	41(67.21)	20(32.79)
	Recurrent	41	18(43.90)#	23(56.10) #
TCC numbers	Single	51	30(58.82)	21(41.18)
	Multiple	51	29(56.86) ◆	22(43.14)◆
TCC size	>2cm	65	36(55.38)	29(44.62)
	<2cm	37	23(62.16) ●	14(37.84)●

★ denotes $p<0.01$ vs G1; △denotes $p<0.01$ vs G2; ★★denotes $p<0.01$ vs Tis~T1;
# denotes $p<0.05$ vs primary; ◆denotes $p>0.1$ vs single; ●denotes $p>0.1$ vs >2cm

Table 2. Relationship between expression of E-CD mRNA and bladder TCC

Index		Case (n)	E-CD/ β-actin
Bladder TCC		24	0.5693±0.3911
Normal bladder tissue		16	1.1205±0.3850 ★
TCC Grade	G1	11	0.8503±0.3964
	G2	9	0.3481±0.1817 ▲
	G3	4	0.2848±0.1369▲#
TCC Stage	Tis~T1	8	0.9623±0.3686
	T2~T4	16	0.3705±0.2168§
TCC occurrence	Primary	15	0.7441±0.3741
	Recurrent	9	0.2739±0.2005 ●
TCC numbers	Single	14	0.5577±0.4676
	Multiple	10	0.5819±0.2746★
TCC size	>2cm	14	0.6106±0.4494
	<2cm	10	0.4078±0.2790◎

★ denotes $P<0.05$ VS TCC; ▲denotes $P<0.05$ VS G1; #denotes $P>0.05$ VS G2; §denotes $P<0.01$ VS Tis~T1; ●denotes $P<0.01$ VS primary; ★denotes $P>0.1$VS single; ◎denotes $P>0.1$ VS >2cm

no significant difference between them ($P>0.1$) (Table 1) .

The expression of E-CD mRNA:

The expression of E-CD mRNA in cases of human transitional cell cancer of bladder and normal bladder mucosa were (0.5693±0.3911) and (1.1205±0.3850) respectively, there was significant difference between them ($P<0.05$); In the cases of G1, G2 and G3, the expression of E-CD mRNA were (0.8503±0.3964), (0.3481±0.1817) and (0.2848±0.1369), there were significant difference in G1 vs G2 and G1 vs G3, ($P<0.05$), but there was not significant difference between G2 and G3 ($P>0.05$). The expression of E-CD mRNA in superficial cases and invasive cases were (0.9623 ±0.3686) and (0.3705 ±0.2168) respectively, there was significant difference between them ($P<0.01$); In the cases of first occurring and recurrence, the expression were (0.7441±0.3741) and (0.2739±0.2005), there was significant difference between them ($P<0.01$); The expression in mono-occuring cases and multi-occuring cases were (0.5577 ±0.4676) and (0.5819 ±0.2746), there was not significant difference between them ($P>0.1$). And the expression in the cases with the dameter above 2cm and those below 2cm is (0.6106 ±0.4494) and (0.4078 ± 0.2790), there was not significant difference between them ($P>0.1$) (Table 2).

DISCUSSION

E-CD, also known as ECAD, Cadherin-1 (CDH1) and Cell-CAM120/80, is a member of a family of transmembrane glycoproteins involved in intercellular adhesion. E-Cadherin is a calcium (Ca^{2+})-dependent protein that binds in a homophilic manner and plays critical roles in epithelial cell-cell adhesion, morphogenesis, and maintenance of tissue architecture [9]. Loss of E-CD expression leads to dissociation of cells from cohesive tissues and correlates with dedifferentiation and invasiveness in a variety of solid tumors, which suggested a role for E-CD as a suppressor of tumor invasion and metastasis [6, 10]. Proteolytic cleavage of E-cadherin from the cell surface may account for the soluble form [11],which has been found in serum [12, 13] and in urine [8] with elevated levels in patients with a variety of cancers. The low expression of E-CD may indicate a high invasion of tumor. In 1991, Frixen et al. found that carcinoma cell lines with an epithelioid phenotype were noninvasive and expressed the epithelium-specific cell-cell adhesion molecule E-cadherin, as visualized by immunofluorescence microscopy and by Western and Northern blotting, whereas carcinoma cell lines with a fibroblastoid phenotype were invasive and had lost E-cadherin expression. Invasiveness of these latter cells could be prevented by transfection with E-cadherin cDNA and was again induced by treatment of the transfected cells with anti-E-cadherin MAbs. These findings indicate that the selective loss of E-cadherin expression can generate dedifferentiation and invasiveness of human carcinoma cells, and they suggest further that E-cadherin acts as an invasion suppressor.[10] In our study, we found that the expression of E-CD had inverse correlation with bladder TCCs grades and stages, which indicates that the low expression of E-CD leads to dissoci-

ation of cells from cohesive tissues, and the tumor cells are prone to invade and metastasize. In clinical practice, E−CD may help assess the invasion and metastasis of the bladder TCC. We also found that tumors size and number had no statistical correlation with the expression of E−CD.

Recurrence is an important feature of bladder TCC, and the high recurrence rate is a chief factor that influences the patients prognosis. E−cadherin is an important determinant of the mechanisms involved in the recurrence rate of bladder cancer [14]. Among the 102 bladder TCC cases in our study, expression of E−CD in the primary group was found significantly higher than that in the recurrence group, so there was a correlation between E−CD and recurrence. In clinical practice, E−CD can be used to assess the possibility of recurrence and more attention and monitoring should be given to the patients of bladder TCC with low expression of E−CD.

In summary, our study has displayed that the expression of E−cadherin in the tissue of bladder TCC is very closely associated with the biological behavior of bladder TCC. This finding will aid us in the early detection and assessment of the clinical course of bladder TCC.

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