Expression of E-cadherin Protein in Transitional Cell Cancer of Bladder and Its Significance

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Abstract **Objective** To study the expression of E-cadherin protein in transitional cell cancer of bladder and its significance. **Methods** E-cadherin was detected by immunohistochemistry SP method in 57 cases of bladder cancer and 7 cases of normal bladder tissues. **Results** Abnormal expression frequency of E-cadherin in G3 group, invasive group were higher than that in G1/G2 group and superficial group ($p<0.05$). The frequency in recurrence group was lower than that in non-recurrence group ($p<0.05$). The 3-year overall survival rate of patients with normal E-cadherin expression was significantly higher than that of the patients with aberrant expression ($p<0.01$). **Conclusion** These results suggest that E-cadherin expression can serve as a recurrence and prognostic indicator for bladder cancer.

Key Words E-cadherin; bladder transitional cell carcinoma; immunohistochemistry

E-cadherin is a transmembrane glycoprotein which mediates a calcium dependent homophilic interaction among epithelial cells. It is critically important for epithelial integrity. The altered expression of E-cadherin selective adhesion molecule have been frequently observed in various tumors. We therefore analyze the expression of E-cadherin in bladder transitional cell carcinoma by immunohistochemical SP method, and investigated the relation between loss or reduction E-cadherin expression and clinicopathological data in bladder cancer.

MATERIAL AND METHODS

Patients and tumor specimens

A total of fifty-seven patients with transitional cell carcinomas of bladder who underwent surgery in Second Hospital of Xi´an Jiaotong University, were included in this study. The group consisted of 45 males and 12 females with a mean age of 58.6 (ranged 30 ~ 80) years old. They did not receive irradiation and anticancer chemotherapy before surgery. The bladder tissue was surgically removed either by transurethral resection (n=25) or by radical cystectomy (n=32). The malignant tumors were classified and graded using UICC-TNM and WHO criteria. The tumor recurred in 2 years after resection were classified as recurrence group. All of patients have well-known clinical follow-up data. The average follow-up time for patients who were still alive at the time of evaluation was 36.5 months (range 3 ~ 68 months). 7 cases of normal bladder samples were collected as normal control.

Methods

Fifty-seven samples in formalin-fixed, paraffin-embedded tissue blocks were serially cut at 4 μm and one section with HE for routine histological examination. E-cadherin immunohistological detection was performed using monoclonal antibody against E-cadherin by immunohistochemical SP method (mouse anti human monoclonal antibody of E-cadherin and SP test kit from Zymed company of united states), Negative controls were duplicate sections similarly stained in which the primary antibody was omitted and replaced by TBS solution.

Evaluation of results

The criteria used for the evaluation of E-cadherin expression was as previously described[1]. Cell with strong brown-yellow membranes stained were determinated as positive. Under 400 times and 5 fields of view were selected continuously. normal (++): ≥90% of the cells positively stained with a high density; abnormal (+/−): <90% of the positive cells or virtually all cells E-cadherin negative.

Statistical analysis

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The correlation of E-cadherin expression with pathological grade, clinical stage and recurrence was evaluated by the X^2 test or Fisher´-test. The evaluation of significance was taken as $p<0.05$. Actuarial survival rate of patients with normal and decreased E-cadherin expression were evaluated according to Kaplan-Meier, and the differences were tested with a log-rank test.

RESULTS

In 7 samples normal urothelium E-cadherin is expressed homogeneously with a typical membranous staining at cell-cell borders (Fig.1)

Correlation between abnormal E –cadherin expression and clinicopathological data of the tumor

Abnormal E-cadherin expression is found in 6 of 27 superficial group and in 23 of 30 invasive group. Likely, it is found in 6 of 18 G1, 10 of 23 G2, and 13 of 16 G3 grade. The frequency of abnormal staining increase with both grade and stage ($p<0.01$). The frequency of abnormal E-cadherin expression in recurrence group was lower than that in non-recurrence group ($p<0.01$). (Table 1, Fig.2, 3)

Relation between E-cadherin **expression and survival**

Kaplan-Meier survival curves show that the 3-year overall survival rate of patients with normal E-cadherin expression was significantly higher than that of patients with aberrant expression (log-rank test $X^2=7.39$, $p<0.01$) (Fig.4)

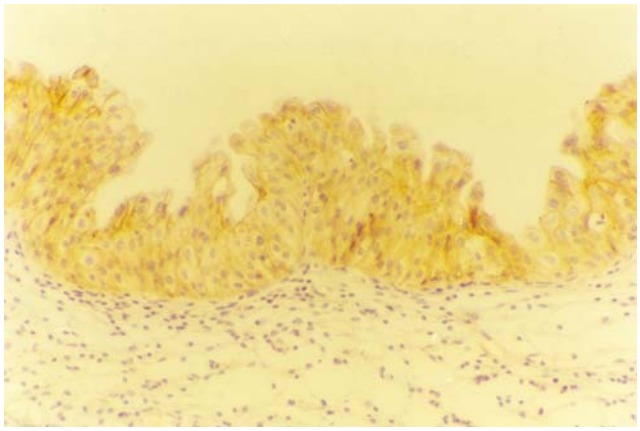

Fig.1 Strong expression of E-cad in normal bladder tissue SP×200

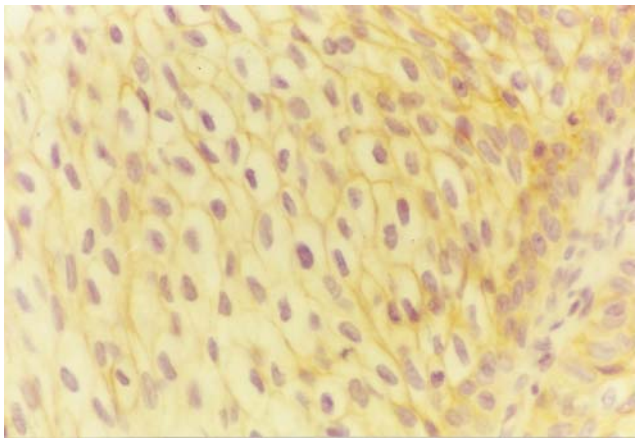

Fig.2 Reduced expression of E-cad in TCC of bladder(++) (T1.G2)SP×400

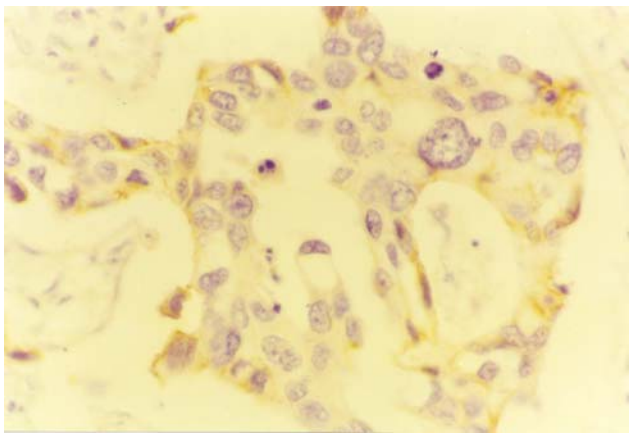

Fig.3 Loss expression of E-cad in TCC of bladder (+) (T3, G3) SP×400

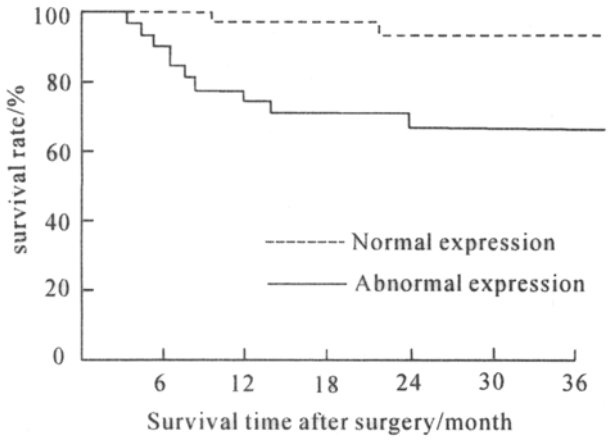

Fig.4 The survival curve of normal and abnormal E-cad expression group

Table 1 The relationship between expression of E-cad and clinical pathology of bladder cancer

Clinical pathology	Case	Expression of E-cad protein		Rate of abnormal expression /%
		Normal expression (++)	Abnormal expression (+/−)	
Superficial group	27	21	6	22.2
Invasive group	30	7	23	76.7
G1	18	12	6	33.3
G2	23	13	10	43.5
G3	16	3	13	81.3
Recurrence group	22	16	6	27.3
Nonrecurrence group	27	9	18	66.7

Superficial group vs. invasive group $P<0.01$; G1 vs .G3 $P<0.05$; G2 vs. G3 $P<0.05$; G1+G2 vs. G3 $P<0.01$; recurrence group vs. Non−recurrence group $P<0.01$

DISCUSSION

Epithelial cadherin (E-cadherin) is a Ca^{2+}-dependent cell-cell selective adhesion molecule that connects cells via homotypic interactions. Its function is critical in the induction and maintenance of cell polarity and differentiation, loss of E-cadherin adhesive function is a critical step in the acquisition of a dedifferentiated and invasive phenotype. Its loss or downregulation is associated with an invasive and poorly differentiated phenotype in a number of human tumor[2,3]. E-cadherin can be considered as an invasion suppressor gene. Its expression or function can prevent tumor cell invasion or progression. Loss of E-cadherin gene or blocking E-cadherin function by anti-E-cadherin mAbs can induce tumor cell invasiveness. The invasiveness of E-cadherin expression negative cell could be prevented by transfection with E-cadherin cDNA and was again induced by treatment of the transfected cells with anti E-cadherin mAbs[4]. In this study, we have investigated E-cadherin protein expression in bladder transitional cell cancer by immunohistochemistry SP method. We found that abnormal E-cadherin expression correlates with both increased grade and stage, this strong correlation seems to indicate that indeed disturbance of E-cadherin expression plays a important role in bladder tumors invasiveness. Because E-cadherin play a major role in the maintenance of intercellular junctions, the abnormal E-cadherin became an critical step in generating dedifferentiation and invasiveness of human carcinomas cells[5]. The loss of E-cadherin expression cells are poorly differentiated and more invasive, have more aggressive biological behavior, so the poor outcome can be reasoned. Our finding show that decreased E-cadherin expression correlates with shorter survival as described by garciadel et al[6]. These results suggest that E-cadherin expression can serve as a prognostic indicator for the biological potential of bladder cancer.

It is interesting to see that E-cadherin normal expression is high in superficial group (77.8%, 21/27) and have high recurrence rate (59.3%, 16/27). The superficial bladder cancer with high recurrence rate is also commonly recognized in urologist clinical observation, but does not in others[7,8]. This results may associated with unique organ structure and physiology of bladder. An important route which lead to tumor recurrences is the shedding of tumor cell with subsequent attachment to damaged or even intact urothelium and formation of new carcinomas at distant sites. In a developed cocultivation model in vitro, E-cadherin play an important role in course of six human bladder carcinomas cell lines attachment to mouse primary urothelium. E-cadherin negative cells did not attach to the primary urothelium and form new carcinomas [9]. The process of shedding of E-cadherin positive tumor cells and their attachment on intact urothelium leading to new tumors elsewhere in the bladder occurs rather frequently. To define the biological significance of E-cadherin, further study is suggested: a) the investigation, evaluation of the function of E-cadherin and the correlation with its regulation factor; b) the examine the molecular mechanisms that regulate E-cadherin gene expression and the transcriptional level.

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