Expression and Significance of p27 and Cyclin E in Bladder Transitional Cell Carcinoma

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Abstract **Objective** To investigate the significance of the expression of p27 protein and cyclin E in the progression and prognosis of bladder transitional cell carcinoma. **Methods** The pathological stage and histological grade were assessed in 121 patients with bladder transitional cell carcinoma . The expression of p27 and cyclin E was tested by immunohistochemical staining. **Results** The expression rate of p27 and cyclin E was 42.1 % and 34.7 % respectively. Both p27 and cyclin E expression were related to histological grade of tumors but not to the pathological stage. A negative correlation was observed between p27 and cyclin E expression ($P<0.05$) . In patients with negative expression of p27 , the 5 years survival rate of the patients with positive expression of cyclin E was lower than those with negative expression of cyclin E ($P < 0.05$). The patients with positive expression of cyclin E and low expression of p27 had a poor prognosis. In patients with positive expression of p27 , the expression of cyclin E had no relationship with the 5 years survival rate. **Conclusion** p27 and cyclin E are important prognostic factors for patients with bladder transitional cell carcinoma.

Key words p27 protein; Cyclin E protein; Bladder transitional cell carcinoma; Prognosis

Both p27 and cyclin E are important cyclins. P27 can combine closely with cyclin E/CD K2, cyclin D/CD K4 and the combo restrains the activity of CD Ks and adjust negatively the cell cycle. The enhancement of p27 expression can prevent the entrance from G1 phase to S phase, so the cell cycle will be stagnated in G1 phase. On the other hand, the decrease of P27 expression can accelerate the cell cycle. Regarded as one of the cyclins in post stage of G1, cyclin E combines with the catalytic subunit CD K2 and participate the process of phosphorylation of Rb protein. The overexpression of cyclin E will accelerate the transition from G1 to S phase. The study has proved that both p27 and cyclin E were closely related with the progression and prognosis of humans malignant tumor. However, the relationship and influence mechanisms between the expression of p27 and cyclins are not clear. Therefore, the expression of p27 and cyclin E in 121 patients with transitional cell carcinoma were tested by immnohistochemistry in order to investigate the significance of the expression of p27 protein and cyclin E in the progression and prognosis of bladder cell transitional carcinoma.

MATERIALS AND METHODS

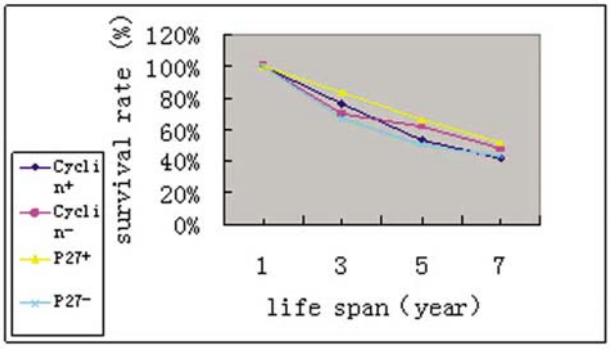
Materials

121 specimen were collected from our academy and the center hospital of Jinan city. Of all the patients who had been confirmed to be bladder cell transitional carcinoma, the number of male is 62 and the female is 59, whose mean age is 61 yeas. The patients had not accepted the chems, radiotherapy and immunotherapy before operation. According to the pathologic grade of tumor tissue, G1 was 41; G2 50; G3 30. In the pathologic stage, 71 samples belong to Ta ~T1, 50 samples belong to T2~T4. We detected the p27 and cyclin E protein in all samples. Another 10 samples of normal bladder tissue were gathered as the control group. All the tissue samples were staged pexyed in 10% formalin, paraffin-embedded and cut sheet in series. The median follow-up phase is 5 years (4~12 years).

Agentia and methods

Mouse anti-human monoclonal antibody of p27 and cyclin E, the Santa Cruz corporations products were bought from Beijing Zhongshan Biotechnology Corporation .The SABC kit and DAB coloration kit were bought from Wuhan Boshide Biotechnology Corporation. The expression of p27 and cyclin E was detected with SABC and the working concentration of first antibody was 1:100. Antigen was repaired by microwave oven. In positive control we use the already known positive slice, and the first antibody was replaced by PBS as negative control.

Fig.1 The Kaplan2 Meier survival curve of the patient with different expression of p27 and cyclin E



Result assessments

According to method in literatures, the positive pigmentation of p27 and cyclin E was located in nuclei, presenting buffy. We selected randomly 10 high power field, counting 1000 tumor cells and the ratio of the positive cell. When the ratio of positive cell ≤20% is regarded as negative and >20% as positive for the the expression of p27; the ratio of positive cell ≤5% is negative, >5% is positive for the expression of cyclin E.

Statistical treatment

To deal with the data with X^2 test, we analyzed the survival rate by Kaplan2Meier method and Wilcoxon test.

RESULTS

The expression of p27 and cyclin E in bladder transitional cell carcinoma tissue

In 121 specimen of the bladder transitional cell carcinoma, the positive expression rate of cyclin E was 34.7% (42/121), and the p27 was 42.1% (51/121). A negative correlation between the expression of p27 and cyclin E (r=0.257, P<0.05) was observed.

The relationship between expression of cyclin E, p27 and the clinical pathological characteristics of bladder transitional cell carcinoma

The expression of p27 and cyclin E had no significant correlation with the clinical staging of bladder transitional cell carcinoma (P>0.05). The expression of cyclinE was significantly related with the tumor histological grade (X^2=5.03, P<0.05). The samples histological grade rised while the expression of p27 weakened or cyclin E rised.

The relationship between prognosis of bladder cancer patient and the expression of p27 and cyclin E

The 5 years survival rate is 53.1% (27/32) in those whose cyclin E is positive, and in those whose cyclin E is negative the rate is 62.3% (20/32), there is no significant difference between them. In patients with positive expression of p27, the five year survival rate is 66.2% (18/27) and it is 50.0%(15/30)in patients with negative

Table 1 The relationship between the expression of P27 and CyclinE and the clinical pathological character of bladder transitional cell carcinoma

Clinical pathological character		N	P27 positive				P27 negative			
			Cyclin E		X^2	P	CyclinE		X^2	P
			+	−			+	−		
Histological grade	G1	41	10	13	0.54	>0.05	2	16	6.05	<0.05
	G2	50	10	9			6	25		
	G3	30	5	4			9	12		
Pathological staging	Ta~T1	71	16	19	0.51	>0.05	5	31	4.35	<0.05
	T2~T4	50	9	7			12	22		

expression. There is no significant difference between them too (P>0.05)(see Fig. 1).

The relationship between the expression of p27 and cyclin E and the clinical pathological character of bladder transitional cell carcinoma

The tumor was divided into two groups: p27 positive group and p27 negative group. Each group was divided into cyclin positive group and cyclin negative group (Table 1). In the p27 negative group, both histological grade and clinical stages of the tumor with the positive expression of cyclin E were higher than the tumor with negative expression of cyclin E. But in p27 positive group,they were no significant difference. The 5 years survival rate was 35.0% in the patient whose expression of p27 was negative and cyclin E was positive, and in the patient with negative expression of both p27 and cyclin E the rate was 67.1%.There was significant difference between them. In the p27 positive group, there was no significant difference of the 5 years survival rate between the cyclin E positive group and cyclin E negative group.

DISCUSSION

The relationship between the expression of p27 and bladder transitional cell carcinoma

Decrease of the protein expression of p27 can lead to many kinds of tumor. The relationship between the decrease of the positive expression of p27 and the increase of the histological grade of the prostate cancer was testified by Guo, et al.[2]. And others had reported that the patients with the lower expression of p27 had higher replase rate and lower the five year survival rate[3]. The results of this study show that the lower expression of p27 is related with the hitological grade in the BTC, the lower expression rate of p27 is, the higher degree of malignancy of the tumor. Therefore, the lower expression of p27 is considered to be related with the development of bladder transitional carcinoma, and the higher malignancy is, the lower expression of p27.

The relationship between expression of cyclin E, p27 and the clinical pathological characteristics of bladder transitional cell carcinoma

The role of cyclin E has not been clearly known in the process of cell transformation and the progression of tumor. Cyclin E is a kind of regulatery protein which can affect on active cyclin E2CDK2 during the G1−S transitional stage.(when cells transite from G1 to S stage). The mechanism how p27 inhibit the activity of CDKs is not very clear,but the combination of p27 and cyclin D2CDK4 decreases the level of p27 which can make cyclin E2CDK2 lose inhibition.The expression of cyclin E can be affected by many factors such as the mutation of gene Rb and gene p16, the overexpression of cyclin D and E2F, and the change of the proteinase pathway. This study proved that the positive expression of cyclin E protein was significantly related with the histological grade (X^2=5.03,P<0.05): the more the expression of cyclin E protein is, the lower the histological grade of bladder carcinoma is, but it is not with clinical stage.

The relationship between the expression of p27 and cyclin E and its signaficance in bladder cancer

p27 is the chief regular factor during the cell cycle, but the relationship between p27 and cyclin E is not very clearly illuminated. Both the enhancement of cyclin E expression and the decrease of positive expression of p27 can increase the activity of CDK2 which leads to the lose of junction point between the G1 and S phase of the cell cycle. The study conducted by Porter et al [4] proved that the decrease of the expression of p27 and the increase of the expression of cyclin E were coincident with the malignant progression and dis−prognosis of breast cancer. Kazuhide, et al [5] also found that the expression of cyclin E was enhanced, and correlated with the expression of p27 in BTC, The result of this study indicated that the expression of p27 had negative correlation with the expression of cyclin E. This result was at equal pace with Kazuhides. Moerover, the enhancement of exogenous cyclin D1or cyclin E can result in the decrese of the expression of p27 in a few cell lines [6]. However, there have been some contrary conclusions. Doki et al.[7] found that the expression of p27 was higher in esophageal carcinoma cell lines than that in normal cells in vitro studies, the trans-

fection of p27 can not inhibite the growth of tumor cells. Sgambato et al [8] found that the enhancement of the expression of cyclin E inhibited the growth of HC11, and increased the expression of p27 acccordingly. Based on the discovery above, it indicated there may be an inhibitive feedback loop between cyclin E and p27. Its function is to keep the balance of positive and negative regulatory factor in G1 phase of cell cycle. Therefore, in the progression of tumor growth, as the result of the decrease of p27, cyclin E expression decreases accordingly to compensate the loss of p27 as the junction during the cell cycle. With the cooperation of other factors, the inhibitive feedback loop is demolished, cyclin E expression is enhanced, thus the tumor become aggravated rapidly. The founding of this study supports this idea. In patients with negative expression of p27, the histological and clinical grade was higher in patients with positive expression of cyclin E than those with negative expression of cyclin E. Monofactorial survival analysis indicated that p27 or cyclin E had no relation with the prognosis merely, but in patients with negative expresison of p27, the five years survival rate was obviously lower in patients with positive expression of cyclin E than those with negative expression of cyclin E. Therefore, it is of great signaficance to detect p27 and cyclin E, and it also can be a valuable index of the prognosis of BTC.

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