Expression of E-Cadherin and the PTEN Gene in Relation to Invasion and Metastasis of Gastric Carcinomas

Xiaoling Li[1]
Yanping Wang[2]
Dongying Wu[2]

[1] Liaoning Provincial Tumor Hospital, Shenyang 110042, China.
[2] The Fourth Laboratory, Cancer Institute, China Medical University, Shenyang 110001, China.

OBJECTIVE To observe the expression of PTEN and E-Cadherin in gastric carcinomas (GCs), and to investigate the relationship between their expression and the pathology and prognosis of patients with GC.

METHODS The proposed markers were detected inmmunohistochemically by using the SABC method in 100 post-operated specimens of GC. The results were statically analyzed by the chi-square and log rank tests.

RESULTS Both E-Cadherin and PTEN proteins were expressed in non-cancerous mucosa. They were reduced or lost in GCs. The abnormal rate of expression of E-Cadherin was 42.0%. The decreased rate of expression in the diffuse-type GC (48.6%) was significantly higher than in the intestinal-type GC (26.7%, $P<0.05$). The abnormal expression of E-Cadherin closely correlated to the depth of invasion ($P<0.05$). The degree of loss of the PTEN protein was 59.0% in GCs. In the diffuse-type GC, the rate of loss of PTEN was (65.7%) which was significantly higher than that in the intestinal-type GC (43.3%, $P<0.05$). The rate of loss of PTEN (64.5%) in GCs with lymph node metastasis was significantly higher than that in GCs without metastasis (41.7%, $P<0.05$). The prognosis of patients with a loss of PTEN protein was worse than the patients with positive expression of PTEN ($P=0.0066$). E-Cadherin was normally expressed in 65.9% of GCs with positive expression of PTEN.

CONCLUSION The loss of E-Cadherin and PTEN markers correlated with infusion and metastasis of GC. The expression of PTEN showed a close relationship to the prognosis of patients. Detection of the 2 markers together aided in the correct prediction of the prognosis of the GC patients and provided information for clinical treatment.

KEYWORDS: PTEN protein, E-Cadherin, gastric carcinoma, invasion and metastasis, prognosis.

Gastric carcinoma is one of the malignant cancers with a high mortality in China. At present, infiltration and metastases are the leading cause of death from this malignancy. Research on understanding the mechanisms of infiltration and metastases of stomach carcinoma and prevention strategies are the most important aspects to improve survival and prognosis for patients. The course of infiltration and metastasis is roughly divided into the following steps. First, cancer cells shed from the primary tumor owing to decreased cell adhesion. This is a prerequisite for tumor metastasis. [1] Second, free

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Email: COCR@eyou.com Tel(Fax): 86-22-2352-2919

cancer cells combine with the extra-cellular matrix through recognition and adhesion to specific antibodies. Third, after adhesion of the cancer cells, hydrolases are released which degrade the extra-cellular matrix. Fourth, cancer cells actively invade blood vessels and form an embolus. Fifth, cancer cells are held in the capillaries of a specific organ where they remain, proliferate and form a secondary carcinoma after passing through the wall of the blood vessels. Metastasis of cancer is a multi-step, continuous and active progress, which is modulated by different oncogenes and tumor suppressor genes.[2]

In this study, we examined the protein expression of E-Cadherin (E-CD), cell adhesion molecules, epithelial cadherin, and the tumor suppressor gene-PTEN in stomach cancer tissues, and related their expression to the mechanism of infiltration, metastasis, pathobiological behavior and prognosis of patients bearing gastric carcinoma, with the aim of finding useful markers to predict potiential metastasis of gastric carcinomas.

MATERIALS AND METHODS

Histological specimens

One hundred surgically removed specimens of gastric carcinoma were retrieved from the pathology files of Liaoning Province Tumor Hospital and Cancer Institute of the China Medical University. The tumors were from 70 males and 30 females with an age range of 26-74 years(mean age: 56 years). Tumor Stages: 6 cases in an early stage; 17 in a medium stage; 77 in an advanced stage. Metastasis: 76 with lymph node metastasis; 24 without metastasis. All cases were followed up for at least 5 years. All gastric specimens were classified according to the WHO histological classification and Lauren's classification criteria. All specimens were fixed in 10% formalin for 18 to 48 h at room temperature and were subsequently paraffin embedded. Four μm thick serial sections were cut.

Immunohistochemistry

Polyclonal antibodies against PTEN (ready for use) were purchased from Maixin Company, Fuzhou. A SABC complex kit was obtained from Boster Company, Wuhan. Known tumor positive slides were used for positive control. For negative controls, tissue sections were incubated with 0.01M PBS instead of the primary antibodies.

Evaluation of PTEN and E-CD

E-CD and PTEN proteins: Clear-brown staining was restricted to the cytoplasm and/or cell membrane, which was considered to be positive for E-CD and PTEN proteins. Expression was defined as follows: *normal expression* (+); positive expression intensity and quantity (more than 90%) in tumor cells similar to the adjacent normal mucosa; *down-regulated expression* (□); tumor cells expressed positively, but relatively less than the adjacent normal mucosa (5% ~90% positive rate), with a decrease in expression intensity; *negative expression*(-): positive rate was less than < 5% or without positive tumor cells.

Statistical analysis

Statistical evaluation was performed using the chi-square test to differentiate between the rates of 2 groups. A P value<0.05 was considered to be statistically significant. The log-rank test was applied to determine the survival rates of all patients with gastric tumors.

RESULTS

PTEN protein and E-CD expression was normally observed to be positive in noncancerous gastric mucosa. PTEN protein expression was less in intestinal metaplasia than in normal mucosa of the stomach both in quantity and intensity, and lower in tumor tissues (59.0%) than in the adjacent normal mucosa. E-CD expression was observed in the cell membrane and cytoplasm in normal gastric mucosa, but only 42.0% in cancerous tissues (down-regulated rate was 27.0% and deletion rate was 11.0%).

The expression of PTEN protein and E-CD in gastric tumor tissues, and their correlation with their histological classification and pathology[3] are shown in Table 1, 2.

Table 1. The relationship between PTEN protein, E-CD expression and the histologic-type of GCs (case, %)

Histologic-type	Cases(n)	PTEN(-)	E-CD(- ~ ±)
Papillary adenocarcinoma	12	8(66.67)	3(25.00)
Well differentiated adenocarcinoma	2	1(50.00)	0(0.00)
Moderately differentiated adenocarcinoma	12	3(25.00)	2(16.67)
Poorly differentiated adenocarcinoma	47	29(61.70)	14(29.79)
Undifferentiated carcinoma	3	2(66.67)	3(100.00)
Ring cell carcinoma	20	15(75.00)	17(85.00)
Mucious adenocarcinoma	4	1(25.00)	3(75.00)
Total	100	59(59.00)	42(42.00)

Table 2. The relationship between PTEN protein, E -CD expression and pathobiological behavior(case, %)

Pathological biobehavior	Cases(n)	PTEN(-)	E-CD(- ~ ±)
Lauren type			
intestinal-type	30	13(43.33)	8(26.67)
diffuse-type	70	46(65.71)*	34(48.57)***
Lymphoid node			
(+)	76	49(64.47)	32(42.11)
(-)	24	10(41.67)**	10(41.67)
Clinical-stage			
early	6	2(33.33)	0(0.00)
middle	17	9(52.95)	5(29.41)
advanced	77	48(62.34)	37(48.05)****

* $P < 0.05$ (χ^2=4.3485)

** P <0.05(χ^2=3.9222)

*** P <0.05 (χ^2=4.1364)

**** P <0.05 (χ^2=5.0336) Early and middle stages compared with advanced stage.

Normal expression of E-CD was found in 65.9% (27/41) of the cases in which the PTEN protein was positive. Of the 52.5% (31/59) of cases in which PTEN was deleted, there was normal expression of E-CD and 64.3% (27/42) of E-CD expression-cases showed abnormal expression.

The log-rank test was applied to examine patient survival following surgery for gastric tumors. As seen in the survival curve (Fig.1), the prognosis of cases with abnormal expression of E-CD was poorer compared to those with normal expression, but there was no significant difference between the 2 groups (P<0.05). Cases with a high expression of PTEN protein survived longer than those with negative expression, resulting in a significant difference (P<0.05, Fig.1).

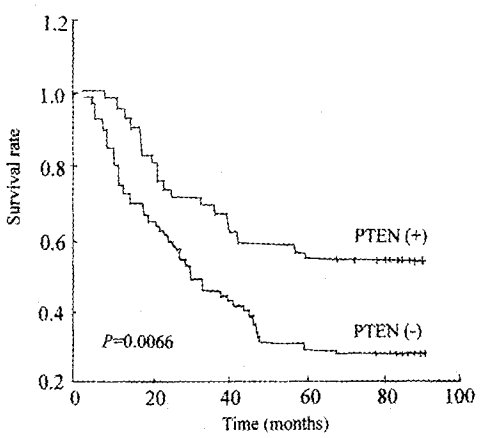

Fig.1. The relationship between PTEN protein expression and prognosis of GCs.

DISCUSSION

E-CD, which is found mainly associated with epithelial cells, is a type of transmembrane glycoprotein that mediates homeotypic cell attachment. It is the key molecule which establishes and maintains the polarity of epithelial cells and mediates the cell-cell tight junction. E-CD and the cytoskeleton protein and actin aid in cellular adhension, thus increasing the expression of calmodulin and enhances the connection between tumor cells and inhibits tumor cells sheding off and metastasizing. An underlying prerequisite for metastasis is that homotypic cells malfunction by loosing the capability to adhere to one another and thus cells shed from the carcinoma. Shino et al.,[4] showed

that there was 32.2 percent of E-CD abnormal expression in gastric carcinoma, and there was a close correlation with the degree of tumor differentiation. Cases with abnormal expression of E-CD had poorer prognosis than those with normal E-CD expression. It was concluded that E-CD is a prognostic marker independent from lymph node metastasis, the depth of infiltration and histological classification.

Mayer et al. [5] found that the decrease of E-CD expression in gastric cancer correlated with the degree of tumor undifferentiation, and with the depth of infiltration in the stomach wall. The low expression of E-CD may predict recurrence of the tumor. Forty-two percent of gastric carcinomas showed abnormal expression (27% in advanced gastric cancer vs. 11% in early gastric cancer); the positive expression rate was 73.3% (22/30) in differentiated gastric carcinoma, which was markedly higher than 51.4% (36/70) in undifferentiated gastric cancer ($P<0.05$). Because a majority of the differentiated gastric cancers were adenocarcinomas, we suggest that E-CD may have an important effect on the adenoid differentiation in gastric carcinomas. In addition, the loss of expression of E-CD correlated with the depth of infiltration ($P<0.05$). We conclud that there is a decrease in the expression of E-CD during the course of infiltration and metastasis. Our study showed no correlation of E-CD expression with the prognosis of patients with gastric carcinomas, but more cases should be studied to further explore the possibility of a relationship.

PTEN (phophatase and tesion homolog deleted on chromosome 10, also called MMAC1/TEP1 gene) is a tumor suppressor gene which was named by Steck et al. and Li et al. in 1997.[6,7] The main structural domain of the PTEN protein is located at the N-terminal. Its protein product-tep1 is a dually-specific phosphatase in the cytoplasm, which dephosphorylates the signal molecule phosphatidylinositol-3, 4, 5-triphosphate (PIP_3), and inhibits abnormal cell proliferation.[8,9] The PTEN protein shares extensive homology with the cytoskeletal proteins auxilin and tension, and combines with actin at the anchor point . The combination of PTEN and the anchor point (including the focal adhesion kinase, Tyr, Src, EGFR, integrin) is involved in the regulation of cell growth, tumor cell infiltration, tumor angiogenesis and metastasis. So far, only a few articles have been published on the relationship between the PTEN protein and gastric cancer. Our results showed that the expression of PTEN protein was significantly lower in gastric cancer tissue than in the normal peripheral gastric mucosa, and the deletion rate of PTEN expression in gastric cancer tissue was higher (59%) than that in normal tissue. We suggest that PTEN protein deletion diminishes its anti-src effect, which prevents inhibition of the phosphorolysis of some intracellular Tyr protein residue, thus transmission of the the intracellular growth stimulus is not broken, as a result, it accelerats the proliferation of gastric cancer cells.

The degree of growth infiltration of gastric cancer is an important pathologic feature and thus is considered to be a characteristic biomarker for the biological behavior of gastric cancer.[10] It was shown that the deletion rate of PTEN protein expression was markedly higher in the diffuse type of gastric cancer compared to that of the intestinal type (65.7% vs 43.3%, $P<0.05$). The deletion rate of PTEN protein expression was significantly higher (64.5%) in gastric cancer with lymph node metastasis compared to tumors without lymph node metastasis (41.7% , $P<0.05$), which indicates that the deletion of the PTEN protein has a close relationship with the diffuse-type gastric cancer and lymph node metastasis. One possibility is that deletion of expression of the PTEN protein could result in inhibition of phosphatase activity and decrease the capability of cell infiltration, metastasis and growth via inhibition of a focal adhension kinase, thus facilitating the tumor cells to infiltrate and metastasize. We conclude that deletion of the PTEN protein expression could be a clinical malignancy phenotypic marker for gastric carcinoma cells. Patients with deletions of the PTEN protein displayed poorer prognosis compared to those with positive expression of PTEN protein. PTEN is proposed as a new molecular biomarker to judge the prognosis of patients with gastric carcinoma.

In our study, 65.9% of the cases with negative expression of PTEN protein normally expressed E-CD; and 52.54% of the cases with deletion of PTEN

normally expressed E-CD. There was no significant difference perhaps due to sampling errors. There seemed to be positive correlation between PTEN and E-CD protein expression. The PTEN protein inhibits abnormal proliferation of cells, regulates cell growth, and exerts an effect on infiltration and metastasis of tumor cells. It could be inferred that the PTEN gene perhaps affects infiltration and metastasis of tumor cells by regulating the expression of E-CD. Decreased or deletion of expression of E-CD might decrease the attachment of the homotypic cells to each other, resulting in the tumor cells sheding from the carcinoma. In relation to the cytoskeleton protein, these 2 biomarkers may exert different functions at different stages of tumor infiltration and metastasis. It may be concluded that combined detection of these 2 biomarkers would be useful in developing a prognosis for patients with gastric carcinoma.

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