PTEN encoding product: a marker for tumorigenesis and progression of gastric carcinoma

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INTRODUCTION
PTEN/MMAC1/TEP1 gene (phosphatase and tensin homology deleted from chromosome ten/mutated in multiple advanced cancer 1/TGF-β-regulated and epithelial cell enriched phosphatase 1) was the firstly defined tumor suppressor which product acted as phosphatase and shared extensive homology with cytoskeletal protein, mapping to human chromosome 10q23.3. PTEN encoding product could not only dephosphorylate the phosphatidylinositol-3, 4, 5-triphosphate (PIP3), but also be involved in cytoskeletal reconstruction and cellular mobility[1-6]. Recently, many studies showed there were several putative mechanisms relating to tumor suppression as follows: inhibiting cell invasion and metastasis by dephosphorylating focal adhesion kinase (FAK); inhibiting cell apoptosis and increasing cell growth by dephosphorylating PIP3; restraining cell differentiation by inhibiting mitogen-activated protein kinase (MAPK) signal pathway[7-9]. Mutation or abnormal expression of PTEN protein occurred commonly in multiple tumors and significantly correlates with tumorigenesis and progression of different malignancies[10-20]. It was reportedly suggested that deletion or mutation of PTEN could enhance the expression of vascular epithelial growth factor (VEGF) and stimulate the proliferation of microvessels in tumor tissues, which in turn closely correlated with tumor invasion and metastasis[21-25].

Gastric carcinoma was one of the commonest malignancies in the world, and even the most frequent in China[26-28]. Although the achievement of early diagnosis and treatment have somewhat improved the patients’ outcome, gastric cancer still remains the major killer among Chinese because the mechanisms of its tumorigenesis and progression were unclear[29]. In this study, we detected the expression of PTEN proteins in gastric cancer and its adjacent noncancerous mucosa, compared PTEN protein expression with its pathologically biological behaviors, and discussed the relationship between the expression of PTEN and VEGF in order to explore the role of PTEN gene product in tumorigenesis and progression of gastric cancer, and to provide scientific foundation for evaluating prognosis of gastric carcinoma.

MATERIALS AND METHODS
Pathology
One hundred and eighty-four cases of surgically removed specimens of gastric carcinoma were collected from Cancer Institute, China Medical University. This study included 102 cases of adjacent normal mucosa, 63 cases of adjacent IM and 6 cases of adjacent dysplasia. According to clinical staging, 37 cases were early, and 147 cases advanced. According to metastasis, 124 cases were accompanied with lymph node metastasis, 6 with liver metastasis (4 of them with lymph node metastasis) and 2 with ovary metastasis. All gastric specimens were classified according to the Lauren’s and WHO’s histological classification criteria.

Immunohistochemistry
All specimens were fixed in 4 % formaldehyde solution,
embedded in paraffin and incised into 5 μm sections. The rabbit anti-human polyclonal antibody against PTEN (ready to use) and mouse anti-human monoclonal antibody against VEGF (ready to use) were purchased from Maxim Biotech. SABC complex kit was from Boster Biotech. For negative control, sections were incubated with PBS (0.01 mol/L, pH7.4) instead of the primary antibodies.

**Evaluation of PTEN and VEGF expression**
Clearly brown staining was restricted to cytoplasm, which was considered as positive for PTEN or VEGF. Slides were scored semi-quantitatively based on staining intensity and distribution. Two pathologists assessed the positive rate according to the percent of positive cells in all counted cells from 5 randomly selected representative fields. The degree of staining was graded in the light of proportion of positive cells as follows: negative (-), positive rate <5 %; weakly positive (+); 5-25 %; moderately positive (++): 25-50 %; strongly positive (+++~++++): >50 %.

**Statistical analysis**
Statistical evaluation was performed by chi-square test to differentiate the rates between two groups. P-value less than 0.05 was considered as statistically significant. SPSS 10.0 software was employed to analyze all data.

**RESULTS**
PTEN was expressed in normal mucosa, intestinal metaplasia, dysplasia and carcinoma of the stomach at the rate of 100 % (102/102), 98.5 % (65/66), 66.7 % (4/6), 47.8 % (88/184), respectively. Dysplasia and carcinoma expressed less frequent than normal mucosa or intestinal metaplasia (P<0.01) (Table 1, Figure 1,2).

|                | n  | PTEN expression | %  |
|----------------|----|-----------------|----|
| “Normal” mucosa| 102| 102             | 0  | 100.0 |
| Intestinal metaplasia | 66 | 65             | 1  | 98.5  |
| Dysplasia       | 6  | 4              | 2  | 66.7  |
| Carcinoma       | 184| 88             | 96 | 47.8  |

Table 1 PTEN expression in normal mucosa, intestinal metaplasia, dysplasia and carcinoma of the stomach

“Compared with “normal” mucosa or intestinal metaplasia, P <0.01 (modified χ²=18.729, 7.115); *Compared with “normal mucosa or intestinal metaplasia, P <0.01 (χ²=80.106, 52.499)

Positive rate of PTEN in advanced gastric carcinoma (AGC) was 42.9 % (63/147), lower than in early one (EGC)(67.6 %, 25/37) (P<0.01). In 124 cases with lymph node metastasis, 50 expressed PTEN protein (40.3 %), whose positive rate of PTEN was higher than those without lymph node metastasis (63.3 %, 38/60) (P<0.01). 41.5 percent of 118 diffuse-type gastric cancers expressed PTEN, less than that of intestinal-type ones (51.8 %, 37/70). Signet ring cell carcinoma expressed PTEN protein at the lowest level (25.0 %, 7/28), more than well and moderately differentiated adenocarcinoma (61.8 %, 21/34) (P<0.01).

None of the gastric normal mucosa showed expression of VEGF, while 75.0 percent of gastric carcinoma expressed it (45/60) (P<0.05) (Figure 3,4). The PTEN-positive cases expressed VEGF at the rate of 78.1 % (25/32), whereas PTEN-negative ones did it at the rate of 71.4 % (20/28). Both rates were not significantly different by statistical analysis (P>0.05).

**Figure 1** PTEN protein was restricted to cytoplasm. It was moderately expressed in normal mucosa (below), while decreased in IM (left top) and dysplasia (right), (20×)

**Figure 2** Well differentiated papillary-tube adenocarcinoma showed weakly positive expression of PTEN protein (40×)

**Figure 3** Mucinous adenocarcinoma of the stomach moderately expressed VEGF (20×)

**Figure 4** SRC showed strongly positive expression of VEGF protein (40×)
Table 2 Relationship between expression of PTEN and the biological behaviors of gastric carcinoma

| Clinopathological staging | PTEN expression |  |  |
|---------------------------|-----------------|---|---|
|                           | n   | +   | ++  | ++++ | -  |
| Early                     | 37  | 25  | 12  | 67.6 |
| Advanced                  | 147 | 63  | 84  | 42.9 |
| Lymph node metastasis     |     |     |     |     |
| +                         | 124 | 50  | 74  | 40.3 |
| -                         | 60  | 38  | 22  | 63.3 |
| Lauren’s classification    |     |     |     |     |
| Intestinal type           | 64  | 37  | 27  | 57.8 |
| Diffused type             | 118 | 49  | 96  | 41.5 |
| Mixed type                | 2   | 2   | 0   | 100.0 |
| WHO’s histological classi|     |     |     |     |
| Papillary adenocarcinoma  | 20  | 10  | 10  | 50.0 |
| Well-differentiated adenocarcinoma | 9 | 5 | 4 | 55.6 |
| Moderated-differentiated adenocarcinoma | 25 | 16 | 9 | 64.0 |
| Poorly-differentiated adenocarcinoma | 85 | 39 | 64 | 45.9 |
| Undifferentiated adenocarcinoma | 5 | 3 | 2 | 60.0 |
| Signet ring-cell carcinoma(SRC) | 28 | 7 | 21 | 25.0 |
| Mucinous adenocarcinoma   | 10  | 6   | 4   | 60.0 |
| Carcinoid                 | 1   | 1   | 0   | -   |
| Squamous cell carcinoma   | 1   | 1   | 0   | -   |

*Compared with early gastric carcinoma, P <0.01(χ²=26.504); a
*Compared with non-lymph node metastasis, P <0.01(χ²=8.580); b
*Compared with intestinal-type gastric carcinoma, P <0.05(χ²=4.416); c
*Compared with well and moderately differentiated gastric carcinoma, P <0.01(χ²=8.380)

DISCUSSION

Deletion or down-regulation of tumor suppressing genes plays an important role in the multiple steps of tumorigenesis and progression of gastric carcinoma. Previous studies on the relationship between alteration of tumor suppressor genes and the development of gastric carcinoma focused on p53\[11,12\], p16\[13\], p27\[14\], p33 (ING1)\[15\], RB\[16\], DCC\[17\] etc. However, few reports were involved in the newly discovered tumor suppressor gene-PTEN in tumorigenesis and progression of gastric carcinoma.

As a tumor-suppressing gene, PTEN makes great contribution to cellular differentiation, reproduction and apoptosis, as well as cellular adhesion and mobility. Some studies showed down-regulation of PTEN protein expression due to genetic changes like mutation, loss of heterozygosity, hypermethylation in gastric cancer, prostate cancer and breast cancer\[2,14,16,19,20\]. Our results showed that decreased expression of PTEN during the courses of normal mucosa→intestinal metaplasia→dysplasia→carcinoma. Gastric dysplasia or carcinoma expressed less PTEN than normal mucosa or intestinal metaplasia (P<0.01), revealing that genetic changes of PTEN gene may play an important role in malignant transition of epithelial cells of gastric mucosa.

Low expression of PTEN gene product was involved in clinopathological stage and metastasis of stomach neoplasms. We found that 42.9 percent of AGC expressed PTEN, less than EGC (P<0.01). Positive rate of PTEN was lower in gastric cancer with than without lymph node metastasis (40.3 % vs 63.3 %, P<0.01). One of the six liver metastases showed negative expression of PTEN in primary or liver metastasis, while the other five cases with liver metastasis showed reduced expression of PTEN protein. These results were similar to other kinds of tumors\[30-36\]. It is suggested that deletion or reduced expression of PTEN protein probably facilitate the metastatic ability of gastric cancer cells. Hwang et al. found that PTEN could enhance mobility and metastasis of tumor cells by regulating matrix metalloproteinases (MMPs) and VEGF\[47\]. There was another report that PTEN dephosphorated FAK so as to be involved in cellular adhesion\[45\]. Deletion or reduced expression of PTEN could result in decreasing cellular adhesion, increasing synthesis of MMPs and VEGF, which subsequently contributed to invasion and angiogenesis of cancer cells. These biological effects possibly underlay prelude of invasion and metastasis of tumor. Our results revealed that reduced expression of PTEN was implicated in progression of gastric cancer probably by decreasing cellular adhesion, increasing cellular mobility and angiogenesis and could act as an objective marker to reflect the biological behaviors of gastric cancer.

In addition, signet ring cell carcinoma showed the lowest expression of PTEN among histological classifications, less than well and moderately differentiated adenocarcinoma (P<0.01), suggesting that decreased expression of PTEN was closely associated with carcinogenesis of signet ring cell carcinoma. Diffuse-type cancer showed less expression of PTEN at the rate of 41.5 % than intestinal-type one. (P<0.05). In this sense, it supported that there were different tumorigenic pathways between diffuse and intestinal-type gastric carcinoma. Diffuse-type gastric cancer, main part of which was signet ring cell carcinoma, showed diffusely invasive growth pattern. It is possible that down-regulation of PTEN could affect the function of cellular skeleton, mobility and adhesion of cancer cells.

Some reports suggested that decreased expression of PTEN encoding product could down-regulate PI3K/AKT pathway, leading to increasing synthesis of VEGF induced by hypoxia inducing factor-1 (HIF-1)\[48-50\]. Our study showed that 75.0 percent of gastric carcinomas expressed VEGF (45/60), significantly more than normal mucosa (0/5) (P<0.05), indicating that VEGF was up-regulated in gastric cancer. But PTEN was down-regulated in gastric cancer. Both PTEN and VEGF showed negative correlation, which was not statistically significant (P>0.05). The relationship between expression of both PTEN and VEGF in tumorigenesis and progression of gastric cancer need proving by amplifying the sample.

In all, loss or reduced expression of PTEN protein occurred commonly in gastric carcinogenesis. Altered expression of PTEN contributed to progression of gastric cancer by increasing cell adhesion, angiogenesis, cell mobility and so on. It was suggested that PTEN could be a useful marker for pathologically biological behaviors of gastric carcinoma. However, the role of PTEN gene and its encoding protein in tumorigenesis and progression of gastric cancer need further investigation.

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