ASSOCIATION OF EXPRESSION OF P53 PROTEIN WITH CELL PROLIFERATIVE ACTIVITY AND PROGNOSIS IN COLORECTAL CANCER

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The expression of p53 protein in 66 cases of colorectal cancer and its relationship to cell proliferative activity, lymph node metastasis as well as prognosis were investigated by means of AB-PAP immunohistochemical technique. The results showed that 62.1% of colorectal cancer was positive of p53 expression. The cell proliferative activity and the frequency of lymph node metastasis in p53-positive tumors were significantly higher than those in p53-negative tumors (P<0.05). The survival rate in patients with p53-positive tumors was significantly poorer than those with p53-negative tumors (P<0.05). Our results suggest that the abnormal expression of p53 and cell proliferation associated with mutations are involved in both human carcinogenesis and lymph node metastasis of colorectal cancer. Examination of p53 expression is of value in understanding the degree of malignancy, and evaluating prognosis of the disease.

Key Words: Colorectal neoplasm, p53 Protein, Proliferative activity

p53 gene, one of well known tumor suppressor genes, has the cell-cycle negative regulating function and regulates cell proliferation.1,2 The mutations and expression of p53 gene in colorectal cancer have been studied comprehensively, but few reports discussed the relationship of its expression to cell proliferative activity, lymph node metastasis as well as prognosis of the disease. We hereby investigated expression of p53 protein and proliferating cell nuclear antigen (PCNA) in colorectal cancer with AB-PAP immunohistochemical technique to find out the correlation between p53 protein expression and cell proliferative activity and prognosis, and evaluate its clinical significance.

MATERIALS AND METHODS

Samples

Sixty-six paraffin-embedded tissues of colorectal cancer (34 cases with lymphatic metastasis) selected from The Department of Pathology, The First Affiliated Hospital, West China University of Medical Sciences were investigated in our study. 11 normal colorectal mucosa were used as control.

Immunohistochemical Study

The immunohistochemical assay for expression of p53 protein and PCNA were performed by means of AB-PAP method as described by Davidoff, et al.3 Anti-p53 protein monoclonal antibody Do-1 (1: 40) and anti-PCNA monoclonal antibody PC10 (1: 50) were obtained from Santa Cruz Company and Zymed Laboratory respectively. ABC and PAP kits were form Dako Company. With each series of tests positive and
negative control were carried out. p53 staining and PCNA staining were considered positive only when definite reddish-brown staining of nuclei was identified. PCNA count was determined by calculating positive cells per 1,000 malignant cells under 40 × light microscope.4

Statistical Analysis

Statistical analysis of data were performed by using chi-square or student’s t-test. Kaplan-Meier survival curves were constructed using the statistical package and assessed using the Logrank test.

RESULTS

Expression of p53 Protein and PCNA in Colorectal cancer

Positive stainings for p53 protein were detected immunohistochemically in 41 out of 66 colorectal cancer (62.1%), which were confined to malignant nuclei, and no membranous and cytoplasmic staining were observed. There were no positive stainings in colorectal normal mucosa. PCNA positive stainings were confined to the nuclei too. PCNA positive cells were present in the lower two-thirds of the crypts in normal mucosa, whereas the positive cells spread diffusely along the glands, even arrived at the surface epithelium in colorectal cancer. The PCNA count was 23.9% ± 10.9% ( ± S) for normal mucosa and 71.2% ± 20.5% ( ± S) for colorectal cancer respectively.

![Graph](image)

Fig 1. Kaplan-Meier Survival Curve

The Relationship between Expression of p53 Protein and Cell Proliferative Activity

It is summarized in Table 1. PCNA count in p53-positive tumors was significantly higher than that in p53-negative tumors (P<0.05), indicating that there was a higher proliferative activity in p53-positive tumors.

Table 1. The relationship between p53 expression and PCNA count

<table>
<thead>
<tr>
<th>p53 expression</th>
<th>No. of cases</th>
<th>PCNA count ( ± S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41</td>
<td>78.2 ± 19.2*</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>61.3 ± 20.1</td>
</tr>
</tbody>
</table>

*P<0.05

The Relationship between Expression of p53 Protein, PCNA and Lymphatic Metastasis

It is presented in Table 2. The p53-positive rate and PCNA count in tumors with lymphatic metastasis were significantly higher than those without lymphatic metastasis. (P<0.05).

Table 2. The relationship between expression of p53, PCNA and lymphatic metastasis

<table>
<thead>
<tr>
<th>Lymphatic metastasis</th>
<th>No. of cases</th>
<th>p53-positive rate(%)</th>
<th>PCNA count ( ± S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>34</td>
<td>25(73.5)*</td>
<td>76.8 ± 21.0*</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>16(50.0)</td>
<td>60.4 ± 20.7</td>
</tr>
</tbody>
</table>

* P< 0.05

The Relationship between Expression of p53 Protein and the Survival of Cancer Patients

The follow-up was made in all cases and succeed in 55 cases (83.3%). Figure 1 shows the Kaplan-Meier survival curves for 55 patients. Patients with p53-positive tumors had a significantly worse survival rate than patients with p53-negative tumors. (logrank test, X² = 5.59, P<0.05).

DISCUSSION

In normal cells, wild-type p53 gene participates in the cell cycle regulation and inhibits the progression of cells from the G1 to the S phase of cell cycle, playing negative regulatory role in the cell proliferation and differentiation.1 It was therefore considered as a modulator of cell proliferation. Wild-type p53 gene