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Immunohistochemical P 53 Protein Expression in Advanced Colorectal Cancer Lesions: No Association with Prognostic Variables or Survival

Yoshiki Tabuchi¹, Takeshi Nakamura², Tetsuya Kuniyasu², Masakazu Ohno², Makoto Usami¹, and Yoshikazu Kuroda²

Correlations of p 53 protein expression in cancer lesions with the already confirmed 13 prognostic variables and survival were examined in 52 advanced (T 2-4) colorectal cancer patients, because the clinical roles of the expression in the cancer lesions remain controversial. The immunohistochemical p 53 protein expression was found in 28 lesions (positive lesions) but not found in 24 lesions (negative lesions). No significant difference between the positive and negative lesions was found in 11 clinicopathologic variables. The carcinoembryonic antigen (CEA) levels (6.7 ± 11.5 and 13.2 ± 18.1 ng/ml) of peripheral and draining venous blood and argyrophilic nucleolar organizer regions (AgNOR) score (3.21 ± 1.21 per nucleus) of the cancer cells in the positive lesions were not significantly different from those (25.1 ± 64.5 and 46.4 ± 114.2 ng/ml, and 3.52 ± 1.10 per nucleus, respectively) in the negative lesions. In addition, no significant difference was also found in the survival curves or the 5-year survival rates of the patients with positive and negative lesions. From these results, it may be concluded that the p 53 protein expression is not associated with the prognostic variables and the prognosis of advanced colorectal cancer patients.

Key Words
Advanced colorectal carcinoma, Immunohistochemical p 53 protein expression, Prognostic variables, Survival.

Introduction
The mutations of p 53 gene, a well-known tumor suppressor gene, have been reported to be closely associated with carcinogenesis and tumor growth of the gastrointestinal.¹-⁷) Regarding the correlations of p 53 gene mutations or protein expression with the already confirmed prognostic variables and prognosis of cancer patients, some investigators have reported that the mutations and protein expression in gastrointestinal cancer lesions correlate with the variables and are clinically used as one of prognosticators.⁸-¹³) On the contrary, other investigators have reported that the gene mutations and/or protein expression are not correlated with the prognostic variables and prognosis or survival.¹⁴-¹⁹) As a result, p 53 gene mutations and/or protein expression in gastrointestinal cancer lesions remain controversial.

In this study, in order to examine the
clinical roles of p53 protein expression in colorectal cancer lesions, the correlations of the immunohistochemical protein expression with the already confirmed 13 prognostic variables and survival were examined in 52 advanced (T2-4) colorectal cancer patients.

Materials and Methods

Fifty-two patients with histologically verified advanced (T2-4 by TNM classification) colorectal adenocarcinoma during the 4-year period between 1984 and 1988 at the First Department of Surgery, Kobe University Hospital (Kobe, Japan) were included in this study. Out of the patients, 44 (84.6%) cases were the distal colorectal (sigmoid colon and rectum) adenocarcinoma cases. The resected specimens were fixed in 10% neutral formalin solution within 48 hours to avoid the reduction of AgNOR staining property20 and then embedded in paraffin. According to the general procedures, the sections were stained with hematoxylin and eosin, and with elastica van Gieson's stain. Eleven clinicopathologic variables including age, tumor location, tumor diameter, gross type, histologic type, depth of cancer invasion into the colorectal wall, grade of lymph node metastasis, liver metastasis, grade of lymph node dissection (D number), histologic stage and Dukes classification were examined by studying 3 sections consisting of a central section of cancer lesions and of 2 parallel sections of the margins with the central section according to the Japanese classification of colorectal carcinoma (first English edition) authorized by the Japanese Research Society for Cancer of the Colon and Rectum.21

The expression of p53 protein and AgNOR score were examined as follows. Four μm sections in parallel with a central section of cancer lesions were made from the paraffin-embedded cancer tissues. The sections were dewaxed and rehydrated, and then endogenous peroxidase was blocked with 3% hydrogen peroxide. Nonspecific bindings were blocked by preincubation with normal bovine serum. The expression of mutant type p53 protein was immunohistochemically examined using a modified avidin–biotin method. As a primary antibody, monoclonal antibody DO–7 (Dakopatts, Glostrup, Denmark) was used at a dilution of 1:100 for 2 hours at room temperature. AEC (3-amino-9-ethylcarbazol) was used as chromogen, and then the specimens were counterstained with hematoxylin. The staining immunoreactivity in the most deeply invasive parts of cancer lesions was examined by two of the authors (Y.T. and T.N.) without prior knowledge of the clinical details, and the lesions were classified into two, negative and positive, lesions according to the immunoreactivity: negative lesions with a little or no reactivity and less than 10% of the reactive cancer nuclei, and positive lesions with at least 10% of the reactive cancer nuclei (Figure 1), as already reported by Cunningham, et al and us.9,22 To evaluate the proliferative activity of the cancer cells, analysis of AgNOR score was performed as already reported by Crocker, et al and us.23,24 In brief, the staining solution, which consisted of 1 volume of 2% gelatin in 1% formic acid and 2 volumes of a silver nitrate solution, was poured over the deparaffinized sections, and the preparations were then left in the dark for 40 minutes at 40 °C. The silver solution was washed off using deionized water, and the sections were dehydrated to xylene and mounted. After color photography
Figure 1. Immunohistochemical stain for p53 protein. The immunoreactivity is found only in the cancer nuclei and in about 30% of the cancer nuclei. In the present study, this lesion was treated as "positive lesion". Original magnification x200.

of the most deeply invasive parts of cancer lesions at the original magnification x200, the number of AgNOR black dots on the trebly enlarged photographs was counted in at least 200 cancer nuclei of all the lesions, and the mean number of the dots per nucleus was defined as the AgNOR score for each of the lesions.  

Immediately after laparotomy, peripheral and draining venous blood was collected from all the patients. Peripheral (p) blood was taken from antecubital venipuncture. Draining (d) blood was collected from the main draining vein of the lesions by inserting a venous catheter, as precisely reported by us.  

The p, d and d-pCEA levels in the patients with positive lesions were not significantly different from those in the patients with negative lesions, although these levels in the latter were somewhat higher than those in the former (Table 2). The means of AgNOR score in the patients with positive and negative lesions were 3.21 and 3.52 per nucleus respectively, and no significant difference was found between them (Table 2).

Survival Curves and the 5-year Survival Rates of the Patients with Positive and Negative Lesions

The survival curve of the patients with negative lesions was not statistically dif-
Table 1. Correlations of p53 protein expression with clinicopathologic variables in colorectal cancer lesions.

<table>
<thead>
<tr>
<th>Clinicopathologic variable</th>
<th>Positive lesion (n = 28)</th>
<th>Negative lesion (n = 24)</th>
<th>Statistics</th>
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<tbody>
<tr>
<td>Age c</td>
<td>63.7 ± 11.1</td>
<td>61.0 ± 11.2</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter (cm) c</td>
<td>5.7 ± 2.0</td>
<td>6.8 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right colon</td>
<td>3</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>left colon</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>rectum</td>
<td>17</td>
<td>14</td>
<td></td>
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<tr>
<td>Gross type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Histologic type</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>well</td>
<td>15</td>
<td>11</td>
<td>NS</td>
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<tr>
<td>moderate</td>
<td>13</td>
<td>11</td>
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<tr>
<td>poor</td>
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<td>Depth of invasion</td>
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<tr>
<td>mp-ss (a1)</td>
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<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>se (a2)</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>si (a1)</td>
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<td>3</td>
<td></td>
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<tr>
<td>Node metastasis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>7</td>
<td>4</td>
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<tr>
<td>Liver metastasis</td>
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<td></td>
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<tr>
<td>0</td>
<td>24</td>
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<tr>
<td>1-3</td>
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<td>D number</td>
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<td>NS</td>
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<td>A-B</td>
<td>6</td>
<td>4</td>
<td>NS</td>
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<tr>
<td>C</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>4</td>
<td></td>
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a The clinicopathologic variables are described according to the Japanese Classification of Colorectal Carcinoma (see Reference 21).
b NS indicates no significance statistically.
c Mean ± standard deviation (SD).
d Dukes D is defined as cancer with distant metastasis.

Table 2. Correlations of p53 protein expression with CEA levels and AgNOR score in colorectal cancer lesions.

<table>
<thead>
<tr>
<th>CEA levels and AgNOR score</th>
<th>Positive lesion (n = 28)</th>
<th>Negative lesion (n = 24)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCEA (ng/ml)</td>
<td>6.7 ± 11.5</td>
<td>25.1 ± 64.5</td>
<td>NS</td>
</tr>
<tr>
<td>dCEA (ng/ml)</td>
<td>13.2 ± 18.1</td>
<td>46.4 ± 114.2</td>
<td>NS</td>
</tr>
<tr>
<td>d-pCEA (ng/ml)</td>
<td>6.6 ± 12.7</td>
<td>21.6 ± 52.9</td>
<td>NS</td>
</tr>
<tr>
<td>AgNOR score c</td>
<td>3.21 ± 1.23</td>
<td>3.52 ± 1.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation (SD).
b NS indicates no significance statistically.
c Dot number per nucleus.
ferent from that of the patients with positive lesions, although the 5-year survival rate of the former was somewhat higher than the rate of the latter (Figure 2).

![Survival curves and the 5-year survival rates of the patients with positive and negative lesions.](image)

**Figure 2.** Survival curves and the 5-year survival rates of the patients with positive and negative lesions. NS indicates statistically no significance.

**Discussion**

In the studies on the correlations of p53 protein expression with the already confirmed prognostic variables and prognosis of cancer patients, some investigators have reported that the expression in gastrointestinal cancer lesions is correlated with the prognostic variables and clinically used as one of prognosticators.8-13 Other researchers, however, have reported that the expression of p53 gene and/or protein is not associated with the prognostic variables and prognosis or survival of cancer patients.14-18 The American Society of Clinical Oncologists has recently concluded that there are insufficient evidence of justify the use of several new markers including p53 protein expression in colon and breast carcinoma.19 As a result, the expression in cancer lesions remains controversial. Therefore, the correlations of immunohistochemical p53 protein expression with the already confirmed 13 prognostic variables and survival were examined in 52 advanced (T2-4) colorectal cancer patients in the present study.

In the current study, no significant difference between the p53 positive and negative lesions was found in any of the clinicopathologic variables strongly affecting survival and/or prognosis. In general, the CEA levels in the blood and the proliferative activity of cancer cells represented by AgNOR score have been already confirmed as the prognosticators of various cancer patients.23-33 Furthermore, some studies have shown that the p53 protein can suppress cell proliferation.3,5,7 Therefore, the association of these variables with the p53 protein expression was also examined in this study. However, no significant difference between the positive and negative lesions was also found in the CEA levels and proliferative activity of the cancer cells represented by AgNOR score. As to the survival curve, the curve of the patients with positive lesions was not statistically different from that of the patients with negative lesions, although the 5-year survival rate of the latter was somewhat higher than the rate of the former. From these results, it may be concluded that the p53 protein expression is not associated with the prognostic variables or the prognosis of advanced colorectal cancer patients, as recently reported by the American Society of Clinical Oncologists.19 The following explanations for these results may be acceptable, although the evidence could not become clear in this study. Namely, it may be reliable that the mutations of p53 gene and/or protein are closely correlated with some steps of carcinogenesis and tumor growth in some
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types of gastrointestinal cancer.\(^{1-7}\) However, it seems that the biological behaviors of cancer itself, especially advanced cancer in which a considerable long time has passed after carcinogenesis, may not be associated with the mutations of p53 gene and/or protein. Furthermore, gastrointestinal carcinogenesis and tumor growth are generally thought to be not only owing to the mutations of p53 gene, but also to many oncogenes including MET, TRK, KS3, HST, E1A, MIC, RAS and such.\(^5\) In fact, it has been reported that gastrointestinal cancer does not show necessarily the mutations of p53 gene and/or protein,\(^2-6,22\) as shown in the present results. Recently, Manne and co-workers have reported that immunohistochemical p53 protein expression is not of prognostic value in the race with primary adenocarcinomas of the distal colorectum.\(^14\) Accordingly, it may be denied that almost all (84.6%) of the patients in this study were the distal colorectal cancer patients. One of the causes that the clinical roles of p53 gene mutations and/or protein expression remain controversial may be due to the difference of the materials and methods among the investigators. Therefore, further precise studies including prospective control studies may still be necessary, in order to completely elucidate the clinical roles of mutations of p53 gene and/or protein in the gastrointestinal cancer lesions.

In conclusion, the correlations of immunohistochemical p53 protein expression in cancer lesions with the already confirmed 13 prognostic variables and survival have been examined in 52 advanced (T2–4) colorectal cancer patients, and the results suggests that the protein expression is not associated with any of the prognostic variables and the prognosis of the cancer patients.

References

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