Prognostic significance of proliferating cell nuclear antigen (PCNA) expression in non-small cell lung cancer.

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Keisuke Aoe**    Shosuke Moriwaki††
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Abstract

Formalin-fixed, paraffin-embedded tissue sections of resected tumors from 90 patients were immunohistochemically studied to assess the prognostic value of proliferating cell nuclear antigen (PCNA) expression in non-small cell lung cancer. The individual tumors were classified into groups of high, moderate or low proliferative grade, and 38 (42.2%) patients had a high grade of proliferation. No statistically significant correlations were observed between PCNA grade and TNM status, pathological stage, resectability, histological type, degree of histological differentiation. Only vascular invasion significantly correlated with proliferative grade (p < 0.05). Survival analysis showed that patients with low proliferative grade tumors survived significantly longer (a 5-year survival rate of 83.3%) than those with high proliferative grade tumors (39.4%, p < 0.005). Cox’s multivariate analysis revealed that PCNA grade was a significant prognostic determinant of survival. These results suggest that PCNA expression provides an independent prognostic variable for patients with non-small cell lung cancer and that it may be useful to consider this factor in treatment planning.

KEYWORDS: proliferating cell nuclear antigen, non-small cell lung cancer, immunohistochemistry, prognostic factor
Prognostic Significance of Proliferating Cell Nuclear Antigen (PCNA) Expression in Non-Small Cell Lung Cancer

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Formalin-fixed, paraffin-embedded tissue sections of resected tumors from 90 patients were immunohistochemically studied to assess the prognostic value of proliferating cell nuclear antigen (PCNA) expression in non-small cell lung cancer. The individual tumors were classified into groups of high, moderate or low proliferative grade, and 38 (42.2%) patients had a high grade of proliferation. No statistically significant correlations were observed between PCNA grade and TNM status, pathological stage, resectability, histological type, degree of histological differentiation. Only vascular invasion significantly correlated with proliferative grade (p < 0.05). Survival analysis showed that patients with low proliferative grade tumors survived significantly longer (a 5-year survival rate of 83.3%) than those with high proliferative grade tumors (39.4%, p < 0.005). Cox's multivariate analysis revealed that PCNA grade was a significant prognostic determinant of survival. These results suggest that PCNA expression provides an independent prognostic variable for patients with non-small cell lung cancer and that it may be useful to consider this factor in treatment planning.

Key words: proliferating cell nuclear antigen, non-small cell lung cancer, immunohistochemistry, prognostic factor

Non-small cell lung cancer represents a mixed group of tumors showing a wide variety of histological properties, clinical courses, and biological expression. The best predictor of clinical outcome is the stage at diagnosis (1, 2). However, great variabilities in outcome within each stage necessitate a search for other prognostic variables to independently distinguish those patients with favorable from those with unfavorable prognosis.

Proliferating cell nuclear antigen (PCNA) is a 36 kilodalton auxiliary protein of DNA polymerase-δ which is maximally elevated in late G1 and S phases of the cell cycle (3, 4), and nuclear PCNA expression has been found in the proliferating compartment of formalin-fixed, paraffin-embedded tissues using the monoclonal antibody to PCNA (5-9). For certain hematological malignancies and solid tumors, PCNA expression has been shown to have an important prognostic value (10, 11). However, the clinical significance of PCNA expression as a prognostic factor is still unclear in surgically treated patients with non-small cell lung cancer. In the current immunohistochemical study, we investigated the correlation of PCNA expression with clinicopathological characteristics and further evaluated the prognostic singificance of PCNA expression in routine histological sections of 90 surgically resected non-small cell lung cancers.

Subjects and Methods

Patients. We evaluated 90 patients who had been diagnosed and surgically treated at Shikoku Cancer Center Hospital between 1983 and 1990. The patients consisted of 63 men and 27 women (average age, 63 years at diagnosis). The histological classification of the tumors was based on the World Health Organization criteria (12) and included 43 adenocarcinomas, 36 squamous cell carcinomas, 5 large cell carcinomas, and 6 adenosquamous cell carcinomas. Three patients who died within one month after surgery or died of intercurrent disease were eliminated from the detailed study, and survival was analyzed in the remaining 87 patients.
Immunohistochemical analysis. Conventional histological sections (4 μm thick) were cut from formalin-fixed, paraffin-embedded materials. The tissue sections were mounted on poly-L-lysine coated glass slides and air dried overnight at room temperature. The sections were dewaxed and incubated for 10 min with normal donkey serum to prevent nonspecific binding of the antibody after blocking endogenous peroxidase by 0.3% hydrogen peroxide in methanol. The avidin–biotin-peroxidase complex (ABC) method was employed for PCNA staining using the ABC kit (Lipshaw Immunon, Detroit, USA). The sections were first incubated with monoclonal IgG antibody against PCNA (diluted 1:25, Novocastra Labs., Newcastle, UK) for 18 h at 4 °C, followed by incubation with the avidin–biotin-peroxidase complex. Labeling was developed with a diaminobenzidine hydrogen peroxide substrate (Wako Pure Chemical Ind., Ltd., Osaka, Japan) and sections were lightly counterstained with 1.0% methyl green. The proportion of PCNA-positive cancer cells was graded as low (0–24%), moderate (25–49%), or high (≥50%) (Fig. 1).

Statistical analysis. The correlation of various disease parameters with PCNA reactivity was analyzed by the chi-square test. Patient survival was calculated from the day of surgery. Survival curves were estimated using the Kaplan–Meier method, and differences in survival were evaluated by the logrank test. The multivariate analyses were performed with the Cox’s proportional hazards model.

Results

Positive staining with anti-PCNA antibody occurred in the nuclei of a variable proportion of cells in non-small cell lung cancers. In general, squamous cell carcinomas showed most of the PCNA-positive cells in the peripheral portions of the tumor nests, whereas PCNA-positive cells were randomly distributed in the adenocarcinomas. Intensity of nuclear staining varied considerably among the PCNA-positive tumor cells.

Of the 90 tumors that were successfully analyzed, 33 (36.7%) had low grade staining, 19 (21.1%) moderate grade staining and 38 (42.2%) high grade staining with anti-PCNA antibody. As indicated in Tables 1 and 2, no statistically significant correlations were observed between PCNA grade and TNM status, pathological stage, resectability, histological type, or degree of histological differentiation. However, there was an inverted correlation between vascular invasion and proliferative grade with a statistical significance (p < 0.05).

The relationship between PCNA grade and postoperative survival was analyzed in 87 selected patients (Table 3 and Fig. 2). When all histological types were evaluated

Fig. 1 Immunohistochemical staining of non-small cell lung cancers with anti-proliferating cell nuclear antigen (PCNA) antibody. Left: Low grade staining in an adenocarcinoma tissue. Middle: Moderate grade staining in an adenocarcinoma tissue. Right: High grade staining in a squamous cell carcinoma tissue (original magnification × 150).
Table 1  Relationship between PCNA reactivity and clinical factors

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Number of patients in different PCNA grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt; 65</td>
<td>21</td>
</tr>
<tr>
<td>≥ 65</td>
<td>12</td>
</tr>
<tr>
<td>T status&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>20</td>
</tr>
<tr>
<td>T2</td>
<td>12</td>
</tr>
<tr>
<td>T3, T4</td>
<td>1</td>
</tr>
<tr>
<td>N status&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>23</td>
</tr>
<tr>
<td>N1</td>
<td>5</td>
</tr>
<tr>
<td>N2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>M status&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>27</td>
</tr>
<tr>
<td>M1</td>
<td>6</td>
</tr>
<tr>
<td>Stage&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Stage I</td>
<td>22</td>
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<tr>
<td>Stage II</td>
<td>3</td>
</tr>
<tr>
<td>Stage III, B</td>
<td>2</td>
</tr>
<tr>
<td>Stage IV</td>
<td>6</td>
</tr>
<tr>
<td>Resectability</td>
<td></td>
</tr>
<tr>
<td>Curative</td>
<td>21</td>
</tr>
<tr>
<td>Noncurative</td>
<td>12</td>
</tr>
</tbody>
</table>

There were no statistical significances between clinical factors (column) and proliferating cell nuclear antigen (PCNA) grades (row).<sup>a</sup>: pathological status; <sup>b</sup>: pathological stages

Table 2  Relationship between PCNA reactivity and histopathological factors

<table>
<thead>
<tr>
<th>Histopathological factors</th>
<th>Number of patients in different PCNA grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>23</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>8</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Degree of differentiation</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>22</td>
</tr>
<tr>
<td>Moderately</td>
<td>9</td>
</tr>
<tr>
<td>Poorly</td>
<td>1</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
</tr>
</tbody>
</table>

There were no statistical significances between histopathological factors (column) and proliferating cell nuclear antigen (PCNA) grades (row) except vascular invasion (<sup>p < 0.05</sup>).

together, patients with low proliferative grade tumors had significantly longer survival times than did those with high grade tumors (<sup>p < 0.005</sup>). The 5-year survival rates were 83.3% for those with low grade tumors, 59.0% for those with moderate grade tumors, and 39.4% for those with high grade tumors. When adenocarcinomas and squamous cell carcinomas were analyzed separately, patients of each type with low grade tumors were found to survive significantly longer than those with high grade tumors (<sup>p < 0.05</sup> and <sup>p < 0.005</sup>, respectively). An analysis of the patients stratified by the resectability of their tumors also showed the same results for those who underwent curative resection (<sup>p < 0.05</sup>) and for those with noncurative resection (<sup>p < 0.05</sup>, not shown). In addition, when analysis was restricted to patients with stage I to II disease, those with low grade tumors lived significantly longer than those with high grade tumors (<sup>p < 0.01</sup>). Similarly patients with stage III to IV disease with low grade tumors survived longer than those with high grade tumors (a 5-year survival rate of 50.0%
versus 8.5%, respectively).

The prognostic significance of PCNA grade, sex, age, pathological stage, resectability, histological type, degree of histological differentiation, and vascular invasion was assessed with Cox’s multivariate analysis (Table 4). Stage of disease (p = 0.0029), histological differentiation (p = 0.0134) and PCNA grade (p = 0.0245) were significant for patients with non-small cell lung cancer.

### Table 4 Multivariate analysis by Cox’s proportional hazards model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>SE</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCNA</td>
<td>1.2308</td>
<td>0.5472</td>
<td>3.42</td>
<td>1.17-10.01</td>
<td>0.0245</td>
</tr>
<tr>
<td>Sex</td>
<td>0.6270</td>
<td>0.4379</td>
<td>1.87</td>
<td>0.79-4.42</td>
<td>0.1522</td>
</tr>
<tr>
<td>Age</td>
<td>0.4845</td>
<td>0.4089</td>
<td>1.62</td>
<td>0.73-3.62</td>
<td>0.2350</td>
</tr>
<tr>
<td>Stage&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6424</td>
<td>0.2160</td>
<td>1.90</td>
<td>1.24-2.90</td>
<td>0.0029</td>
</tr>
<tr>
<td>Resectability</td>
<td>0.8828</td>
<td>0.5379</td>
<td>2.24</td>
<td>0.84-6.94</td>
<td>0.1008</td>
</tr>
<tr>
<td>Histology</td>
<td>0.0740</td>
<td>0.3841</td>
<td>1.08</td>
<td>0.53-2.20</td>
<td>0.8389</td>
</tr>
<tr>
<td>Differentiation</td>
<td>1.1556</td>
<td>0.4571</td>
<td>3.18</td>
<td>1.27-7.93</td>
<td>0.0134</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>0.0009</td>
<td>0.4841</td>
<td>1.10</td>
<td>0.42-2.83</td>
<td>0.8510</td>
</tr>
</tbody>
</table>

<sup>a</sup>: pathological stage; SE: standard error; CI: confidence interval. PCNA: See Table 1.

### Discussion

Several immunohistochemical methods, including bromodeoxyuridine labeling (13), Ki-67 staining (14), and DNA polymerase-α staining (15), have been used to assess cell proliferation in human tumors. PCNA staining has an advantage over other techniques because it can be used with routinely processed formalin-fixed, paraffin-embedded tissues. PCNA staining has been shown to have significant clinical and pathological correlations in
hepatocellular carcinomas (7), neuroblastomas (8), colorectal carcinomas (9) and acute leukemias (16). Recently, Woods et al. (10) reported a good correlation between PCNA grade and histological grade of tumor, and also a significant relationship between PCNA grade and S + G2 + M phase fraction by flow cytometric analysis in gastrointestinal lymphomas. A few studies (17, 18) showed high PCNA grade in lung cancers, but the clinical significance remains undefined. Our current study demonstrated that PCNA expression was associated with vascular invasion and was also prognostically significant in non-small cell lung cancer.

The heterogeneity of PCNA staining within a given tumor suggests that more than one area should be sampled to test for PCNA expression. Heterogeneity may reflect different growth rates or other phenotypic heterogeneity within tumors, which might be caused by variable tumor blood supply or cellular phenotypic instability (19). The finding of heterogeneity of PCNA expression within a given tumor might, for example, be predictive of partial versus complete response to growth rate-dependent antitumor therapy.

Our major objective in this study was to evaluate the prognostic value of PNCA expression. Survival analysis showed that higher reactivity of PCNA significantly correlated with a shorter survival time in these patients. A similar association between high PCNA expression and poor prognosis in malignant lymphomas (10) and hemangiopericytomas (11) was reported previously. Analysis of the patients stratified by stage of disease showed that, of those with stage I to II disease, the cases with high PCNA grade had significantly worse prognoses; a similar tendency was noted for those with more advanced disease. Moreover, the significant correlation between PCNA grade and patient survival was independent of the resectability of tumors. PCNA grade was a significant factor in multivariate analysis for survival, with stage of disease and degree of histological differentiation for all patients.

We conclude that PCNA expression, determined immunohistochemically using formalin-fixed, paraffin-embedded tissues, is an important and stage-independent prognostic variable of survival of patients with non-small cell lung cancer and that it should be considered in treatment recommendations.

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References


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