RELATIONSHIP BETWEEN PROLIFERATING CELL NUCLEAR ANTIGEN EXPRESSION AND ITS MALIGNANCY POTENTIAL IN COLORECTAL CARCINOMA

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ABSTRACT

Objective: To study the relationship between proliferating cell nuclear antigen expression and its malignancy potential in colorectal carcinoma. Methods: Paraffin sections of 86 patients with advanced colorectal carcinoma were assessed by immunohistochemical study, using a mouse monoclonal antibody (pc-10, DAKO Co. USA) to check proliferating cell nuclear antigen (PCNA). To compare PCNA with conventional clinicopathologic factor, including p53 overexpression, tissue carcinoembnyonic antigen immunoreactivity pattern and flow cytometric DNA ploidy for assessing tumor malignancy potential. In addition, recurrence and survival of patients with advanced colorectal carcinoma after curative resection were analyzed in accordance with degree of PCNA expression. Results: PCNA-labeling index (PCNA-LI) increased significantly as the tumor stage advanced (p=0.0001). Strong correlations were observed between PCNA-LI and various pathologic parameters, including histologic differentiation (P=0.0027), lymphatic invasion (P=0.0001), vascular invasion (P=0.0001), lymph node metastasis (P=0.0001), and liver metastasis (P=0.0036). Mean PCNA-LI was also significantly higher in tumor with DNA aneuploidy (P=0.0006) and negative (P=0.01). Linear relationships were demonstrated between PCNA-LI and clinical outcomes; Recurrence rate was significantly greater in the group with higher than the mean PCNA-LI, who underwent curative resection ($P<0.01$), and three-year survival rates for curative cases with higher than the mean PCNA-LI were significantly poorer than those with lower than mean PCNA-LI ($P<0.005$). Conclusion: There were correlations between PCNA-LI and various pathologic parameters, PCNA-LI increased significantly as the tumor stage advanced in colorectal carcinoma, the rates of recurrence and death got higher as PCNA-LI increased after curative resection for colorectal carcinoma.

Key words: Colorectal carcinoma, Proliferating cell nuclear antigen, and Malignancy potential, pathologic factors

Surgical resection of the tumor is the best treatment option for colorectal carcinoma; some patients receiving curative resection die from the cancers because of recurrences of local, regional, or distant metastatic focus. Until now, pathologic variable has been widely used to determine whether adjuvant therapy is to be performed and to predict a likelihood of long-term survival.[1,2] Cellular proliferation is a fundamental biologic activity that may be useful in understanding the biologic behavior of tumors. In recent years, research on colorectal cancer has focused on identifying the relationship between malignant potential of neoplasms and cellular proliferative activity.[3] Proliferating cell nuclear antigen (PCNA) is an acidic nuclear protein, and its expression is necessary for DNA synthesis.[4,5] Its functions act as an auxiliary protein for DNA polymerase-δ in DNA synthesis.[6,7] PCNA has been found to be a useful marker in immunohistochemical analysis of cell kinetics because its expression and distribution correlate with the rate of cell proliferation and DNA synthesis.[8,9] We undertook this study with the aim to assess the correlation between cellular proliferative activity and malignant potential in colorectal carcinomas.

MATERIALS AND METHODS

Patients

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This study included a total of 86 patients with advanced primary colorectal carcinomas, 51 men and 35 women; mean age 57 years (range: 38-76 years). All patients underwent colorectal carcinoma surgical resection in our hospital from 1986 through 1993. None received preoperative chemotherapy or radiation therapy, and no patients with synchronous other disease. All patients were followed-up after resection.

Methods

Histologic sections from all 86 surgical specimens were reviewed. An average of five sections per tumor specimen was prepared for PCNA immunostaining, and paraffin sections were routinely stained with hematoxylin and eosin. Histologic differentiation based on the World Health Organization's classification and staging according to the Astler-Coller staging system was determined. Orcein-stained sections were used for detecting vascular invasion. Samples were also taken from the same tissue blocks for flow cytometric study of tumor cell DNA. To assess the prognostic effect of PCNA expression, other immunohistochemical studies of p53 expression of pathologic details including primary tumor site, lymph node and liver metastasis, and lymphatic and vascular invasion. Concrete process as shown following: Tissue blocks containing the deepest invasive tumor margin from the material fixed in formalin and embedded in paraffin were retrieved for immunohistochemical staining of PCNA expression. The acidin-biotin-peroxidase complex technique was used. Four-micron-thick sections were cut, mounted on glass slides, and air-dried overnight at room temperature. Sections were dewaxed in xylene, followed by rehydration in graded alcohol series, and incubated with 3 percent hydrogen oxide for 15 minutes to block endogenous peroxidase activity. Subsequently, sections were washed in phosphate-buffered saline, and 10 percent normal nonimmune rabbit serum was applied for 20 minutes to reduce nonspecific antibody binding. PC-10 (a mouse monoclonal antibody) was diluted 1:50 and reacted with tissue specimens at room temperature for two hours. Sections were subsequently washed with phosphate-buffered saline, and immunohistochemical staining was performed using the avidin-biotin-peroxidase complex. Diamino-benzidine was used as a chromogen, and sections were counterstained with Mayers' hematoxylin.

Criteria for Immunohistochemical Assessment

PCNA expression at the invasive margin was interpreted by the labeling index (LI), which was defined as mean percentage of stained cancer cells per 1,000 tumor cells in a minimum of five randomly chosen microscopic fields (original magnification, ×200). P53 expression was scored qualitatively as positive when staining was visible in any nuclei within the tissue section. CEA immunoreactivity was considered positive when reddish brown precipitate was present within tumor cells.

RESULTS

Nuclear staining readily identified PCNA-positive brownish nuclei of tumor cells. Considerable intratumor heterogeneity was observed in the distribution of stained tumor cells in this study. Mean PCNA-LI of the invasive tumor margin was 46.65, with a range of 13.33 to 80.64, $\bar{x} \pm s$ was 46.65 ± 16.60.

Correlation between PCNA-LI and various clinicopathologic factors are shown in Table 1. There was no statistically significant association between PCNA-LI and anatomic tumor location, however, significant differences existed in relation to Astler-Coller stage, histologic grade, lymph node metastasis and liver metastasis, lymphatic and vascular invasion. There was a trend for PCNA-LI to become significantly higher when the pathologic stage in terms of Astler-Coller system increased (p=0.0001) and histologic differentiation became poorer (p=0.0027). Mean PCNA-LI in patients with lymph node metastasis was significantly higher than in those without metastasis (p=0.0001), PCNA-LI for 25 tumors with lymphatic invasion was significantly higher than that of 61 tumors with out lymphatic invasion (p=0.0001). Similar results were obtained on relationships between vascular invasion and liver metastasis and PCNA-LI (p=0.0001 and 0.0036, respectively)

PCNA-LI in relation to flow cytometric and other immunohistochemical findings as shown in Table 2. There was no statistically significant difference between PCNA-LI and p53 expression. DNA ploidy, however, was significantly related to expression of PCNA; mean PCNA-LI in DNA aneuploid patients was 53.72 in comparison with 40.88 in diploid patients (p=0.0006). With comparison to tissue carcinoembryonic antigen (CEA) immunoreactivity, PCNA-LI was high in patients with negative immunostaining (52.62±13.86, $\bar{x} \pm s$) and low in patients with positive immunostaining (45.48±16.12). There was a statistically significant difference between these groups (p=0.01).

Except for 11 patients with stage D disease who received palliative treatment, 75 patients underwent curative resection of colorectal carcinoma. Seventy-six patients were stratified into two subgroups: high PCNA-LI was greater than mean value; low PCNA-LI

Table 1. The Relationship between PCNA-LI and clinicopathologic variables

Variable	No. of cases	PCNA-LI ($\bar{x} \pm s$)	*P* Value
Astler-Coller stage			
B1	6	19.93±6.24	0.0001
B2	26	31.76±8.40	
C1	9	50.00±8.14	
C2	34	55.17±9.63	
D	11	66.60±10.22	
Histologic grade			
Well	47	31.76±8.40	0.0027
Moderate	25	51.20±17.69	
Poor	14	55.80±12.14	
Tumor site			
Right	17	41.76±17.02	0.2956
Transverse colon	7	55.41±12.13	
Left colon	16	48.82±15.76	
Rectum	46	46.19±17.09	
Lymph node metastasis			
Negative	54	29.54±9.23	0.0001
Positive	32	56.64±10.66	
Lymphatic vessel invasion			
Negative	61	40.52±15.18	0.0001
Positive	25	56.64±10.66	
Vascular invasion			
Negative	58	40.20±14.44	0.0001
Positive	28	59.70±12.69	
Liver metastasis			
Negative	79	44.92±16.04	
Positive	7	64.97±11.28	0.0027

Table 2. PCNA-LI in relation to flow lytometric and immunohistochemical variables

Variable	No. of cases	PCNA-LI ($\bar{x} \pm s$)	*P* Value
Tumor ploidy			
DNA diploid	48	40.88±16.39	0.0006
DNA aneuploid	38	53.72±14.05	
CEA immunoreactivity			
Positive	73	45.48±16.12	0.01
Negative	13	52.62±13.86	
P53 immunoreactivity			
Positive	51	45.27±17.96	0.41
Negative	35	48.42±14.44	

group, whose index was less than the mean. Recurrence rate in low PCNA-LI group was 15.4 percent (6 of 39), whereas that in the high group was 41.7 percent (15 of 36), which was significantly higher than that in the former group ($P<0.01$) as shown in Table 3.

Table 3. Relationship between recurrence after colorectal carcinoma curative resection and PCNA-LI

PCNA-LI grade	Curative operative	Recurrence(%)
High group	36	41.7(15/36)*
Low group	39	15.4(6/39)*

*$P<0.01$

Kaplan-Meier survival curves for the high and low PCNA-LI groups of patients as shown in Figure 1. Of 86 cases, prognosis was poorer in 46 patients with high PCNA-LI, and their three-year survival rate was 41.6%, but three-year survival rate was 78.2% in 40 cases with low PCNA-LI. It had a significant difference between the high and the low groups ($P<0.005$). Three-year survival rate was 80.2% in 39 patients with low PCNA-LI who underwent curative resection. However, prognosis was poorer in 36 patients with high PCNA-LI, their three-year survival rate was 53.2%. There was also a significant difference between the two groups ($P<0.01$).

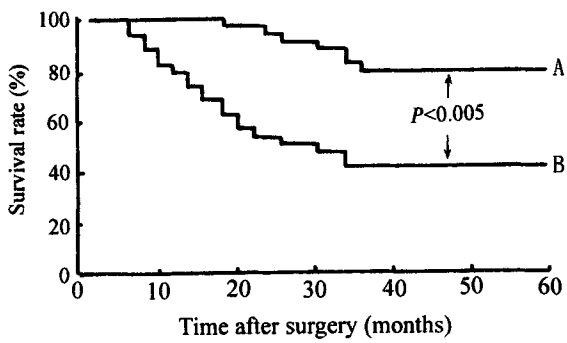

Fig. 1. Prognosis of the low PCNA-LI group (A) appeared to be significantly better than that of high PCNA-LI group (B) ($P<0.005$).

DISCUSSION

Cell kinetic information obtained from immunohistochemical techniques has the particular advantage of maintaining spatial orientation of proliferating cells within tissue.[10] To assess the information of cell proliferation kinetics, we have performed immunohistochemical study using paraffin-embedded material. Because of intratumor heterogeneity, careful consideration should be paid to pathologic sections that are to be prepared in the immunohistochemical study of PCNA expression. On the basis that more aggressive malignant clones appear during development of multiple clonal subpopulations,[11] and that relevant portion of the

tumor with histologic heterogeneity is the area of deepest tumor invasion,[12] we evaluated the relationship between PCNA expression and prognostic parameters of the invasive tumor margin rather than the tumor site. This study clearly indicates that colorectal carcinomas with a higher PCNA-LI at invasive tumor margins were associated with aggressive biologic behavior and metastatic potential, and strong statistical correlations were found with histologic grade, lymph node metastasis, lymphatic and vascular invasion, and liver metastasis. Our data suggest that PCNA-LI may be a useful indicator of proliferative activity and progression of colorectal carcinoma. We also performed additional studies of cell proliferative activity and tumor malignancy potential using flow cytometry, immunohistochemical CEA, and p53 stains. Quirke, et al.[13] considered that prognosis was poor in patients with aneuploid tumor or high proliferative activity. Our study showed significantly higher PCNA-LI in cancers with DNA aneuploidy and in those with a cytoplasmic CEA staining pattern or negative staining; however, no correlation was found between PCNA-LI and p53 expression. These results suggest that PCNA-LI reflect cellular proliferative activity and malignant potential. Although some authors have reported no association between Dukes stage and tumor cell proliferation,[13,14] this study shows a linear relationship between Astler-Coller stage and PCNA-LI. This discrepancy may imply studying the importance of the tumor area, as tumors advance into the deeper layer of the colonic wall gradually, clones in the invasive portion of these tumors rather than at the predominant tumor site would be expected to be more malignant biologically. Three-year survival data would be significant enough to assess prognosis because most deaths caused by recurrence in patients with colorectal carcinoma occur within three years after surgery. Our results showed a significantly higher recurrence rate and poorer prognosis in the higher than mean PCNA-LI group, which indicated that PCNA-LI could be considered valuable as a prognostic predictor. To sum up, there were correlations between PCNA-LI and various pathologic parameters, PCNA-LI increased significantly as the tumor stage advanced in colorectal carcinoma, the rates of recurrence and death got higher as PCNA-LI increased after curative resection for colorectal carcinoma.

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