DNA PLOIDY AND p53 EXPRESSION ASSOCIATED WITH TUMOR SITE AND LYMPH NODE METASTASIS IN COLORECTAL CANCER

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ABSTRACT

Objective: To study the association of DNA ploidy abnormality and p53 overexpression with the carcinogenesis of colorectal cancer. Methods: DNA ploidy and p53 expression were measured in a series of 42 colorectal adenocarcinomas by means of flow cytometry and immunohistochemical test. Results: 17 tumors (40%) were diploid and 25 (60%) aneuploid. The aneuploid tumors were significantly more common in the distal colon than in the proximal colon ($P<0.01$). Aneuploidy was significantly associated with lymph node metastasis ($P<0.05$). There was no correlation between DNA ploidy and the other clinicopathological variables. Of the 22 samples examined, the positive rate of p53 expression was 59% (13/22). p53 expression was more frequently observed in the distal tumors (11/13) than in the proximal tumors (2/9) ($P<0.05$). Conclusion: Our data support the hypothesis that the carcinogenesis of colorectal cancer might differ in proximal and distal tumors. DNA ploidy abnormality and p53 overexpression may play an important role in the development of distal colorectal cancer.

Key words: Colorectal cancer, DNA ploidy, Flow cytometry, p53

The development of human colorectal cancer is characterized by a variety of genetic alterations. Genetic changes associated with colorectal cancer have been investigated intensively in recent years. p53 gene mutation is one of the most common genetic alterations in colorectal tumors and is linked to cellular proliferation and genetic instability.[1] A relationship between the carcinogenesis of colorectal cancer and p53 gene and DNA ploidy pattern also has been investigated widely.[2-5] p53 gene mutation and lymph node metastasis were demonstrated to be more frequent in DNA aneuploid than in DNA diploid colorectal tumors. These observations suggest that DNA ploidy abnormality and p53 gene mutation may influence the development and progression of colorectal cancer. In this study, we investigated DNA ploidy and p53 expression in a series of 42 colorectal adenocarcinomas by means of flow cytometry and immunohistochemical testing to determine the association of DNA ploidy and p53 overexpression with the carcinogenesis and biological features of colorectal cancer.

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MATERIALS AND METHODS

Patients

Excised primary colorectal cancer was obtained from 42 patients treated at Beijing Institute for Cancer Research, from 30 to 77 years old, 19 males and 23 females. Specimens were frozen in liquid nitrogen immediately after surgery until use. According to the tumor site, 18 tumors were located in the proximal colon (ascending and transverse colon) and 24 in the distal colon (descending, sigmoid colon and rectum).

Flow Cytometric DNA Ploidy Analysis

Tumor samples were thawed at room temperature and minced with scalpels in phosphate-buffered saline (PBS). The cell suspensions obtained were filtered through a 160 μm nylon mesh to remove large cell clumps and debris and washed twice with PBS and then fixed in 70% ethanol at 4°C overnight. After washing, the cell concentration was adjusted to approximately 1×10^6/ml and the samples were stained for 30 minutes at

4°C in the dark with propidium iodide (50 μg/ml). Before flow cytometric analysis, the samples were passed through a 50 μm nylon mesh. The DNA content was measured with an EPICS-PROFILE II flow cytometer (Coulter). The data were analyzed using muticycle software. The degree of DNA ploidy was determined by the DNA index (DI). The tumors were considered to be DNA diploid (DI=1.0±0.05) and DNA aneuploid (DI≠1.0±0.05).

Immunohistochemical Staining

The tissue sections were deparaffinized routinely and rehydrated through xylene and graded alcohol series. After incubation in a microwave oven, the sections were washed in PBS, and 10% normal horse serum was then applied for 30 minutes to reduce nonspecific antibody binding. Staining of p53 protein was performed using the murine monoclonal antibody DO-1 (Santa Cruz, CA). Sections were incubated with the primary antibody (1:100 dilution) overnight at 4°C in a moist chamber. After PBS rinses, biotinylated horse antimouse immunoglobulin G was applied for 30 minutes. Slides were reacted with avidin-biotin peroxidase (1:100 dilution) complex for 30 minutes and washed with PBS. Peroxidase activity was visualized as a reddish-brown color change after 0.03% hydrogen peroxide and 0.05% diaminobenzidine treatment. The sections were counterstained with hematoxylin. Negative controls were obtained by the complete reaction without the DO-1 antibody. Nuclear staining of more than 15% of tumor cells was classified as p53-positive.

Statistical Analysis

The x^2 test was used for statistical analysis.

RESULTS

Of the 42 tumors studied, 17 (40%) were classified as DNA diploid and 25 (60%) as DNA aneuploid. DNA ploidy status was related closely to the tumor site and the lymph node metastasis (Table 1). The aneuploid tumors were significantly more common in the distal colon than in the proximal colon ($P<0.01$). Aneuploidy was significantly associated with lymph node metastasis when compared with diploidy ($P<0.05$). There was no correlation between DNA ploidy status and patient age, sex, TNM stage, or degree of differentiation ($P>0.05$). Of the 22 samples examined by immunohistochemistry, the positive rate of p53 expression was 59% (13/22). A relationship also could be found between p53 expression and tumor site. p53 expression was more frequently observed in the distal tumors (11/13) than in the proximal tumors (2/9) ($P<0.05$).

Table 1. DNA ploidy status in relation to clinicopathological variables

Variables	No. of patients	Diploid	Aneuploid
Sex			
Male	19	9 (47%)	10 (53%)
Female	23	8 (35%)	15 (65%)
Ages (yrs)			
<60	22	8 (36%)	14 (64%)
≥60	20	9 (45%)	11 (55%)
Tumor site			
Proximal colon	18	12 (67%)	6 (33%)
Distal colon	24	5 (21%)	19 (79%)
TNM stage			
I–II	18	10 (56%)	8 (44%)
III–IV	24	7 (29%)	17 (71%)
Node status			
Positive	20	4 (20%)	16 (80%)
Negative	22	13 (59%)	9 (41%)
Differentiation			
Well/Moderate	29	11 (38%)	18 (62%)
Poor	13	6 (46%)	7 (54%)

DISCUSSION

The difference of biological behavior between tumors of the proximal and distal colon has been demonstrated. Molecular genetic studies showed that 17p and 18q allelic deletion and p53 gene mutations were more frequent in tumors of the left colon and rectum than those in the right colon.[6, 7] In this study, DNA aneuploidy and p53 protein overexpression were found to be more common in the distal tumors than in the proximal tumors. It has also been reported that DNA aneuploidy tumors showed a higher frequency of p53 gene mutations.[4] The precise mechanism and significance are not well understood, but these findings may reflect that distinct mechanisms are involved in the development of carcinomas at different sites of the colon and rectum. They may also explain a biological feature difference between proximal and distal tumors. Our study also shows that DNA aneuploidy is significantly associated with lymph node metastasis when compared with DNA diploidy. There was no correlation between DNA ploidy status and patient age, sex, or TNM stage and histologic differentiation. This result suggests that DNA aneuploidy pattern might be associated with a trend of metastasis of the tumor. This would support the hypothesis that DNA aneuploidy pattern correlates with aggressive tumor behavior, and thus may be regarded as a prognostic factor in colorectal cancer.

Analysis of DNA content by flow cytometric technique has been used to evaluate the prognosis in patients with colorectal carcinomas. Although its prognostic significance in colorectal cancer remains unconfirmed, most investigators agree that patients with DNA diploid tumors had a better survival when compared with patients with DNA aneuploid tumors.[8, 9] The clinical value of DNA ploidy abnormality and p53 overexpression in colorectal tumors is still unclear. Studies of different genetic alterations in larger samples of tumors will hopefully be of use in the diagnosis and management of colorectal cancer.

In conclusion, the present results indicate that DNA ploidy status and p53 expression are associated with some clinicopathological variables, such as tumor site and lymph node involvement. These findings support the hypothesis that the carcinogenesis of colorectal cancer might differ in proximal and distal tumors. DNA ploidy abnormality and p53 overexpression may play an important role in the development of distal colorectal cancer.

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