

## P21 and CEA expression and AgNOR counts in dimethylhydrazine-induced colon carcinoma in rats

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### Abstract

**AIM:** To study P21 and carcinoembryonic antigen (CEA) expression and to measure argyrophilic nucleolar organizer region (AgNOR) counts in various lesions of colonic mucosa and the mechanism of carcinogenesis.

**METHODS:** Thirty-eight male Wistar rats were injected with dimethylhydrazine (DMH) once a week for 25 wk. P21 and CEA expression was detected by immunohistochemical methods, and AgNOR was counted by silver staining paraffin sections from various colonic lesions.

**RESULTS:** The incidence of colonic carcinoma in DMH-treated rats was 71.05%(27/38), and lymph node metastasis occurred in six rats. Immunohistochemical studies showed that P21 was primarily expressed in dysplasia and carcinomas, while CEA was expressed in carcinomas and metastatic tumors. AgNOR counts were higher in dysplasia and carcinomas. There were significant differences in P21 and CEA expression between benign and malignant lesions ( $P < 0.05$ ). The difference in AgNOR counts was also significant between normal and dysplastic tissues, and between dysplasia and malignant lesions ( $P < 0.05$ ).

**CONCLUSION:** Dysplasia is a premalignant change of colonic carcinoma. The detection of P21 *via* immunohistochemistry and AgNOR counting may be an important clinical screening technique for colon carcinoma and premalignant lesions.

**Key words:** Colonic neoplasms; Carcinoembryonic antigen; Ras oncogene; Nucleolus organizer region; P21 oncogene

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### INTRODUCTION

The transformation-inducing genes most frequently detected in solid human tumors are members of the Ras family of oncogenes. It has been suggested that P21 overexpression in the early neoplasm stage may play an important role in carcinogenesis<sup>[1,2]</sup>. In this study, we examined P21 and CEA expression and quantified nuclear silver stain of the granules of nucleolar organizer region (AgNOR) in 1.2 dimethylhydrazine (DMH)-induced colonic carcinoma in rats to study the carcinogenesis mechanism.

### MATERIALS AND METHODS

#### Animals

Fifty male Wistar rats weighing approximately 170 g were randomly divided into a test group (T group,  $n = 40$ ) and control group (C group,  $n = 10$ ). Rats in the T group were subcutaneously injected with 20 mg/kg body weight DMH once a week. Rats in the C group rats were given 0.9% NaCl solution at the same time. DMH administration lasted 20 wk. At the 10<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup> week, one rat was sacrificed in each group, and 10 were sacrificed at the 20<sup>th</sup> week. The rest were continually fed with standard diet until the 25<sup>th</sup> week, when they were all sacrificed. Intestinal tissues were fixed in 10% neutral formalin.

#### Pathological and immunohistochemical examination

Tissue specimens were collected from the tumor, adjacent tissues and normal colonic mucosa in T group rats, and normal colon mucosa were collected from the C group. Serial paraffin sections were cut at 4  $\mu$ m thickness and were stained with hematoxylin and eosin (HE). The ABC immunohistochemical method was used for P21 staining, and the PAP method was used for CAE staining. The working dilution of the anti-P21 monoclonal antibody (Huamei Company) was 1:40, and 1:100 for the anti-CEA antibody (DAKO Company). PBS was used as a negative control.

#### AgNOR staining technique

A one-step silver staining method was used for AgNOR detection. The observation method and statistical standard were performed as

**Table 1 DMH (dimethylhydrazine)-induced carcinogenesis in rats**

Lesions	<i>n</i>	Incidence (%)	Number of mass
Well-differentiated adenocarcinoma	17	44.74	20
Undifferentiated adenocarcinoma	3	7.89	5
Mucinous adenocarcinoma	7	18.42	9
No tumor	11		
Total	38	71.05	34

**Table 2 Histological changes in colons of DMH (dimethylhydrazine)-treated rats**

Histological types	T group	C group
Normal epithelial cell	0	6*
Inflammation	10	4
Dysplasia	10	0
Adenoma	3	0
Carcinoma	34	0

\*With normal mucosa for all intestines.

**Table 3 The distribution of positive P21 and Carcino Embryonic Antigen (CEA) immunohistochemical staining in rat colonic lesions**

Histological types	<i>n</i>	p21 (%)	CEA (%)
Benign			
Normal epithelial cell	10	0 (0.0)	0 (0.0)
Adenoma	3	1 (33.3)	0 (0.0)
Dysplasia	10	6 (60.0)	3 (30.0)
Malignant			
Well-differentiated adenocarcinoma	20	14 (70.0)	16 (80.0)
Undifferentiated adenocarcinoma	5	1 (20.0)	4 (80.0)
Mucinous adenocarcinoma	9	7 (77.8)	6 (66.7)

*P* < 0.05 for p21 and CEA between benign and malignant lesions.

described previously<sup>[3]</sup>.

## RESULTS

### Animal experiments

Of the 38 rats in the T group (two rats unexpectedly died during the experiment and were excluded), 27 developed colonic carcinoma with a total of 34 masses. The incidence of carcinogenesis was 71.05% (27/38). Various colonic mucosal lesions are summarized in Table 1. The tumors were 1-13 mm in diameter and more frequently occurred in the distal colon. Most tumors appeared nodular in shape. Six rats developed mesenteric lymph node metastasis, and two developed gastric and pulmonary metastases.

### Histopathological changes

T group rats exhibited inflammation and atypical dysplasia of the colonic mucosa at the 10<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup> week (Figures 1 and 2). We observed adenomas and adenocarcinomas after 20 wk with metastasis (Table 2). We never observed dysplasia or tumor development in the control group.

### Immunohistochemical staining

P21 and CEA staining of colonic lesions is summarized in Table 3. Normal and inflammatory mucosa were negative for expression. P21 was weakly expressed in adenomas and primarily expressed in dysplasia and carcinoma (Figure 3), while CEA was expressed in dysplasia, adenocarcinoma and metastasis (Figure 4). The difference in P21 and CEA expression between benign and malignant colonic mucosal lesions in DMH-treated rats was statistically significant (*P* < 0.05).

### AgNOR count

The AgNOR counts in various colonic mucosa lesions were as follows: adenoma 2.7 ± 0.18, dysplasia 3.2 ± 0.21, and adenocarcinoma 4.6 ± 0.26, and normal epithelial cell 1.45 ± 0.08. In colonic adenocarcinoma, the AgNOR granules not only increased in number, but also became larger and irregular. The AgNOR counts were statistically significantly different between normal mucosa and dysplasia, as well as dysplasia and malignant tissue (*P* < 0.05).

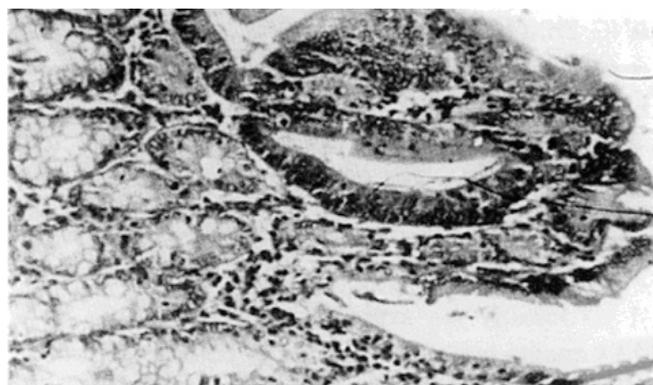


Figure 1 Dimethylhydrazine (DMH)-treated rats, dysplasia localized to the upper 1/3 of the colonic mucosa. HE × 100

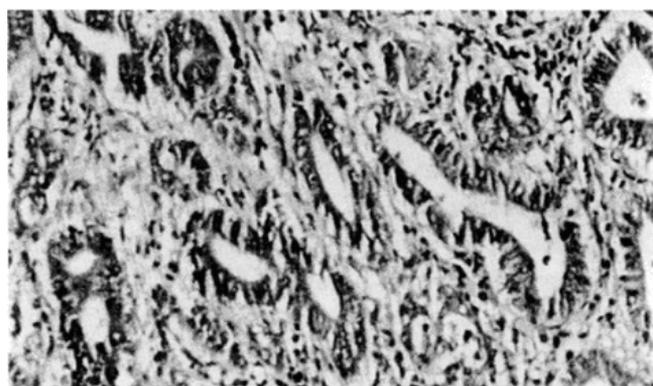


Figure 2 Dimethylhydrazine (DMH)-treated rat, colonic adenocarcinoma. HE × 100

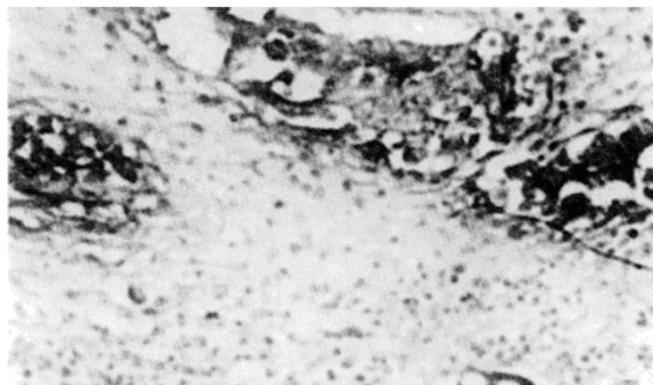


Figure 3 Colonic adenocarcinoma, positive P21 expression in the invasive carcinoma nest. ABC method × 200

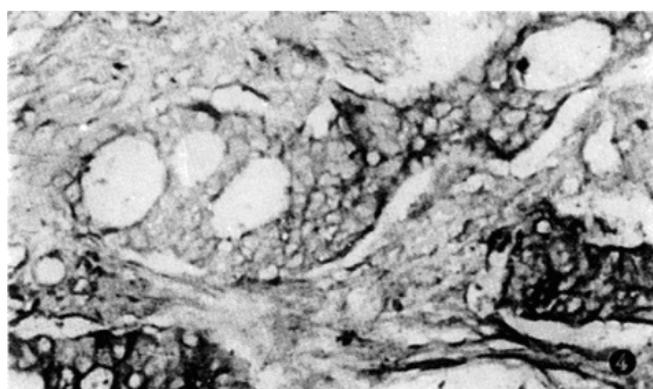


Figure 4 Colonic adenocarcinoma, positive CEA expression in the invasive carcinoma nest. PAP method × 200

However, among the various colonic carcinomas, the differences were not statistically significant (*P* > 0.05).

## DISCUSSION

We treated rats with DMH to induce colonic carcinoma and observed the sequential changes in the colonic mucosa. The marked dysplasia

in the epithelial cells initially involved one or two glands and was localized to the upper 1/3 of the mucosal layer (Figure 1). As the experiment progressed, the number of atypical glands increased, and mucosal noduli formed. Cell heterogeneity also increased, and ultimately led to colonic adenocarcinoma with invasion and metastasis, strongly suggesting that dysplasia is a premalignant lesion for colonic carcinoma.

The activation of oncogenes and P21 overexpression might be critical for malignant cell transformation<sup>[4]</sup>. P21 can inhibit the intercellular signal transmission, leading to loss of controlled cell mitosis. Therefore, P21 overexpression is an important sign of carcinogenesis<sup>[5]</sup>. In our study, we found that P21 protein was expressed in both dysplasia and adenocarcinoma. These data suggest that Ras may exert its effects on cell growth as early as the dysplasia stage to further promote cell transformation. The results support the hypothesis that dysplasia is a premalignant lesion of colonic carcinoma; therefore, ras P21 expression could be an early diagnostic indicator for premalignant lesions. CEA expression is another major marker in colonic carcinoma diagnosis. In DMH-induced colon carcinoma, dysplasia presented with P21 overexpression and increased AgNOR counts, while CEA was only expressed in carcinoma and metastasis. In metastasis, the AgNOR

granules increased and clustered, which is a typical indicator of the malignant colonic carcinoma phenotype. We suggest that P21 and CEA are two proteins expressed in colon carcinoma at different stages of differentiation. In conclusion, P21 immunohistochemical detection and AgNOR quantification important for clinical screening of colon carcinoma and premalignant lesions.

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