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Expression of *ras* Oncogene p21 Product in Malignant and Benign Lesions and Normal Mucosae of the Stomach in China

Chang-Shan REN[1], Hong CHEN[1], Noboru KUZUMAKI[2], Toshiharu YAMASHITA[3], Masanobu SHINDOH[3] and Kei FUJINAGA[3]

[1] *Department of Molecular Biology, Cancer Research Institute, China Medical University, 5 duan, 3 hao, Najing jie, Hepin-qu, Shen-Yang, China,*

[2] *Laboratory of Molecular Genetics, Cancer Institute, Hokkaido University School of Medicine, North 15, West 7, Kita-ku, Sapporo 060,*

[3] *Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College, South 1, West 17, Chuo-ku, Sapporo 060, Japan.*

ABSTRACT

Expression of c-*ras* oncogene was analyzed in 75 cases of carcinomas, 38 benign lesions and 22 normal mucosae of the stomach in Chinese subjects by use of the monoclonal antibody rp28, which reacts to the c-Ha-*ras* and c-Ki-*ras* p21 products. The incidence of p21 positive cases (more than 20% of total cells stained by rp28) was 90.7% in gastric carcinomas, 10.5% in benign gastric lesions and 4.5% in normal mucosae. While 34/75 gastric carcinomas contained strongly rp28-reactive (++) cells, none of the benign lesions or normal mucosae did. The mean percentage of rp28 reactive (+ or ++) cells in each group was 52.2% in gastric carcinomas, 6.3% in benign lesions and 2.3% in normal mucosae. These indicate that *ras* p21 expression is significantly high in the gastric carcinomas compared to the benign lesions and normal mucosae of the stomach. Tests of tubular adenocarcinomas with different degrees of differentiation showed significantly higher amounts of p21 product in well differentiated (71.7%) and moderately differentiated (67.3%) samples than in poorly differentiated adenocarcinomas (40.9%), which contained more, in tern, than mucinous adenocarcinomas (39.2%) and undifferentiated carcinomas (27.5%). This suggests that Ha- and/or Ki-*ras* p21 expression may have some roles in the maintenance of the glandular structure in gastric carcinomas.

Key words: Gastric carcinoma, *ras* p21 expression, Monoclonal antibody

INTRODUCTION

So far, more than 50 kinds of oncogenes have been isolated from tumorigenic retroviruses and by the DNA transfection of cancer cell DNA to NIH3T3 cells

(1). Among them, the activated form of the *ras* oncogene has most frequently been found from various human cancers including bladder, mammary and pulmonary carcinomas and neuroblastoma (1). The *ras* gene family is composed of Ha-*ras*, Ki-*ras* and N-*ras*; acquisition of oncogenic potential (activation) occurs as a result of point mutation at codons 12, 13 or 61 (2). The *ras* oncogene codes a protein of 189 amino acids with a molecular weight of 21 kilodaltons (kd) and thus is called p21. Copious expression of *ras* oncogene p21 product has been reported in mammary carcinomas (3), prostatic cancers (4) and gastric cancers (5, 6). In this study, the reactions to the anti-*ras* p21 monoclonal antibody rp28 were compared in 75 carcinomas, 38 benign lesions and 22 normal mucosae of the stomach in China. The relationship between the *ras* p21 expression level and the biological characteristics and pathomorphology of gastric carcinomas is also discussed.

MATERIALS AND METHODS

Tissue specimen.

Tissue specimens of carcinomas, benign lesions and normal mucosal tissues of the stomach were obtained from 75 patients who consulted the First, Second or Third Affiliated Hospitals of China Medical University from 1985 through 1988. The cancer specimens were composed of 6 papillary adenocarcinomas, 30 tubular adenocarcinomas (15 well differentiated and 15 moderately differentiated ones), 17 poorly differentiated adenocarcinomas, 12 mucinous adenocarcinomas and 10 undifferenciated carcinomas. The 38 benign gastric lesions were composed of 6 dysplasias, 5 gastric polyps which are not further classified here into adenomatous, regenerative or other types, 23 chronic gastritis samples (10 with prominent intestinal metaplasia and 13 without metaplasia) and 4 chronic gastric ulcers. The tissue specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin, then processed for histopathological and immunohistochemical investigation.

Immunohistochemical study.

Rp28 is a monoclonal antibody which reacts to p21s encoded by c-Ha-*ras* and c-Ki-*ras* genes derived from mice and humans (7). Immunohistochemical staining was performed according to the ABC method (8). Briefly, after tissue sections were deparaffinized and processed with alcohol, they were treated with methanolic hydrogen peroxide followed by 1% normal bovine serum. Then, tissue sections were immersed in rp28 (1/100 dilution), incubated at 37℃ for an hour and washed with PBS. Biotinylated second antibody and ABC reagents

(Vector Laboratory, CA, USA) were subsequently applied. Specific antigens were visualized using 0.01% H_2O_2 and 0.05% diaminobenzidine tetrahydrochloride (DAB) in 0.05 M Tris-HCl (pH 7.6). We divided the p21 expression by three criteria, according to the intensity of the reactions to the antibody in the respective tissues. In positive (+) cells, the reaction products were distributed sparsely or localized to one or a few well-defined site(s) in the cytoplasm, strongly positive (++) cells appeared dark-brown throughout the cytoplasm, and negative (−) cells possessed no obvious reaction products within the cytoplasm. Blank control was made by replacing the first antibody rp28 with 0.9% NaCl; otherwise, the same procedure was followed. The percentage of positive cells was determined by counting the proportion of cells positive for rp28 staining in samples of 200 to 400 cells in each specimen.

RESULTS AND DISCUSSION

The incidence of rp28 reactive cells in each tissue was determined by the criteria described in the Materials and Methods (Fig. 1). Positive cases (more than 20% of total cells + or ++) were found in 68 out of 75 cases of cancers (90.7%), 4 out of 38 cases of benign lesions (10.5%) and 1 out of 22 cases of normal mucosae (4.5%) (Fig. 1, Table 1). Thirty-four and 37 samples of the 75 gastric cancer tissues, respectively, contained some highly-positive (++) and positive (+) cells (Table 2). Although some positive cells were found in 18 of the 38 benign lesions and 5 of the 22 normal mucosae, highly positive cells were never found there (Table 2, Fig. 2, A). Among the benign gastric lesions, some cases of dysplasia contained larger amounts of p21 than other benign lesions did (Fig. 1). There were also marked differences in the rp28 reactivity between malignant and benign gastric lesions and normal mucosae; the mean percentages of positive cells were 52.2%, 6.3% and 2.3%, respectively (Table 3). The malignant tissues clearly showed ($p<0.01$) higher reactivity of rp28 than benign lesions and normal mucosae of the stomach.

The cellular reactivity of rp28 with gastric cancers was further analyzed in

Table 1 *Incidence of p21 positive cases in carcinomas, benign lesions and normal mucosae of the stomach*

Tissues	Number of cases	Number of positive cases[1)]	Incidence (%)
Carcinomas	75	68	90.7
Benign lesions	38	4	10.5
Normal mucosae	22	1	4.5

[1)] More than 20% of total cells counted had reacted to the rp28 (+ or ++).

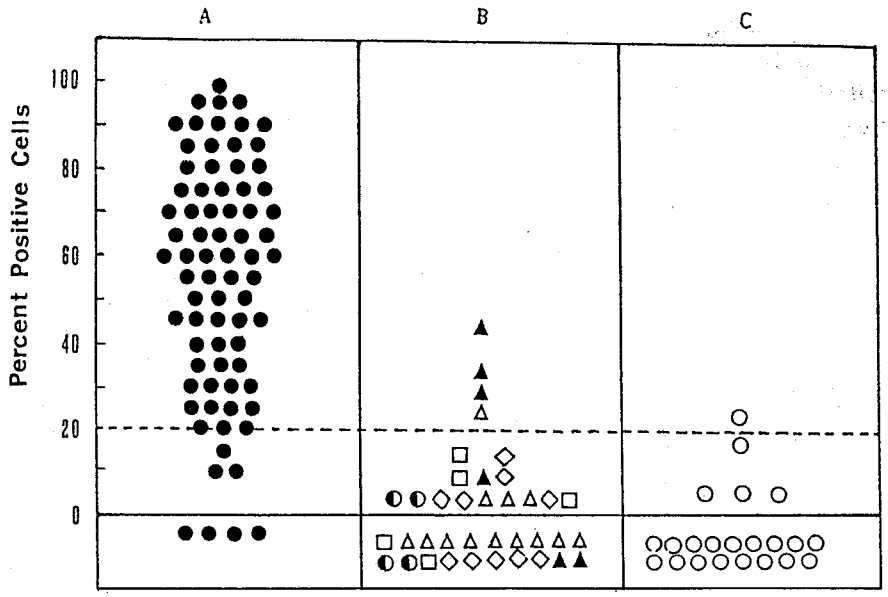

Fig. 1 Percentage of reactivities of rp28 with malignant, benign, and normal tissues of the stomach. Each symbol represents a different individual. A, carcinomas (●); B, benign lesions, dysplasia (▲), gastric polyps (□), chronic gastritis (△), chronic gastritis with intestinal metaplasia (◇) and gastric ulcers (◐); C, normal mucosae (○).

Table 2 *Reactivity of the rp28 with carcinomas, benign lesions and normal mucosae of the stomach*

Tissues	Number of cases tested	Number of cases with staining intensity of +	++	Incidence of p21 positive cases
Papillary adenocarcinoma	6	2	4	6/6
Well differentiated tubular adenocarcinoma	15	4	11	15/15
Moderately differentiated tubular adenocarcinoma	15	6	9	15/15
Poorly differentiated adenocarcinoma	17	11	5	16/17
Mucinous adenocarcinoma	12	8	3	11/12
Undifferentiated carcinoma	10	6	2	8/10
Benign gastric lesions	38	18	0	18/38
Dysplasia	6	4	0	4/6
Gastric polyps	5	3	0	3/5
Chronic gastritis	13	4	0	4/13
Chronic gastritis with intestinal metaplasia	10	5	0	5/10
Gastric ulcers	4	2	0	2/4
Normal mucosal epithelial cells	22	5	0	5/22

Table 3 *Mean percentage of positively stained cells with rp28 in carcinomas, benign lesions and normal mucosae of the stomach*

Tissues	Number of cases studied	Mean percentage of positive (+ or ++) cells[1)]
Carcinomas	75	52.2±28.7[2)]
Benign lesions	38	6.3±10.7
Normal mucosae	22	2.3± 5.7

[1)] X^2 test : $p<0.01$ between every pair of groups
[2)] Mean±SD

Table 4 *Mean percentage of positively stained cells with rp28 in gastric cancers*

Histopathological classification	Number of cases studied	Mean percentage of positive (+ or ++) cells
Well differentiated tubular adenocarcinomas	15	71.7±29.6[1)]
Moderately differentiated tubular adenocarcinomas	15	67.3±19.4
Poorly differentiated adenocarcinomas	17	40.9±29.5
Mucinous adenocarcinomas	12	39.2±25.5
Papillary adenocarcinomas	6	68.3±19.9
Undifferentiated carcinomas	10	27.5±27.8

[1)] Mean±SD

relation to the degree of histological differentiation (Table 4). Well and moderately differentiated tubular adenocarcinomas contained larger number of p21-expressing cancer cells (67.3-71.7%) than poorly differentiated adenocarcinomas (40.9%). Undifferentiated carcinomas contained the fewest cancer cells reactive to rp28 of all the types of gastric carcinomas. The representative features of well differentiated tubular adenocarcinoma strongly stained by rp28 are shown in Fig. 2, B.

Our immunohistochemical results indicated that cells of gastric cancers contained significantly larger amounts of p21 product than those of benign lesions and normal mucosae. Since rp28 reacts to both the c-Ha-*ras* and c-Ki-*ras* gene products (7), our immunohistochemical procedure measured the total amount of c-Ha- and c-Ki-*ras* p21. Studies of *ras* p21 expression by immunohistochemistry and *in situ* hybridization using c-Ha-*ras*, c-Ki-*ras* and N-*ras* probes revealed that overexpression of p21 protein in gastric adenocarcinomas had resulted from c-Ha-*ras* transcription (5). This study leaves open the question whether the 4 of the 75 gastric cancers which failed to react to rp28 developed in spite of low expression of Ha- and/or Ki-*ras*.

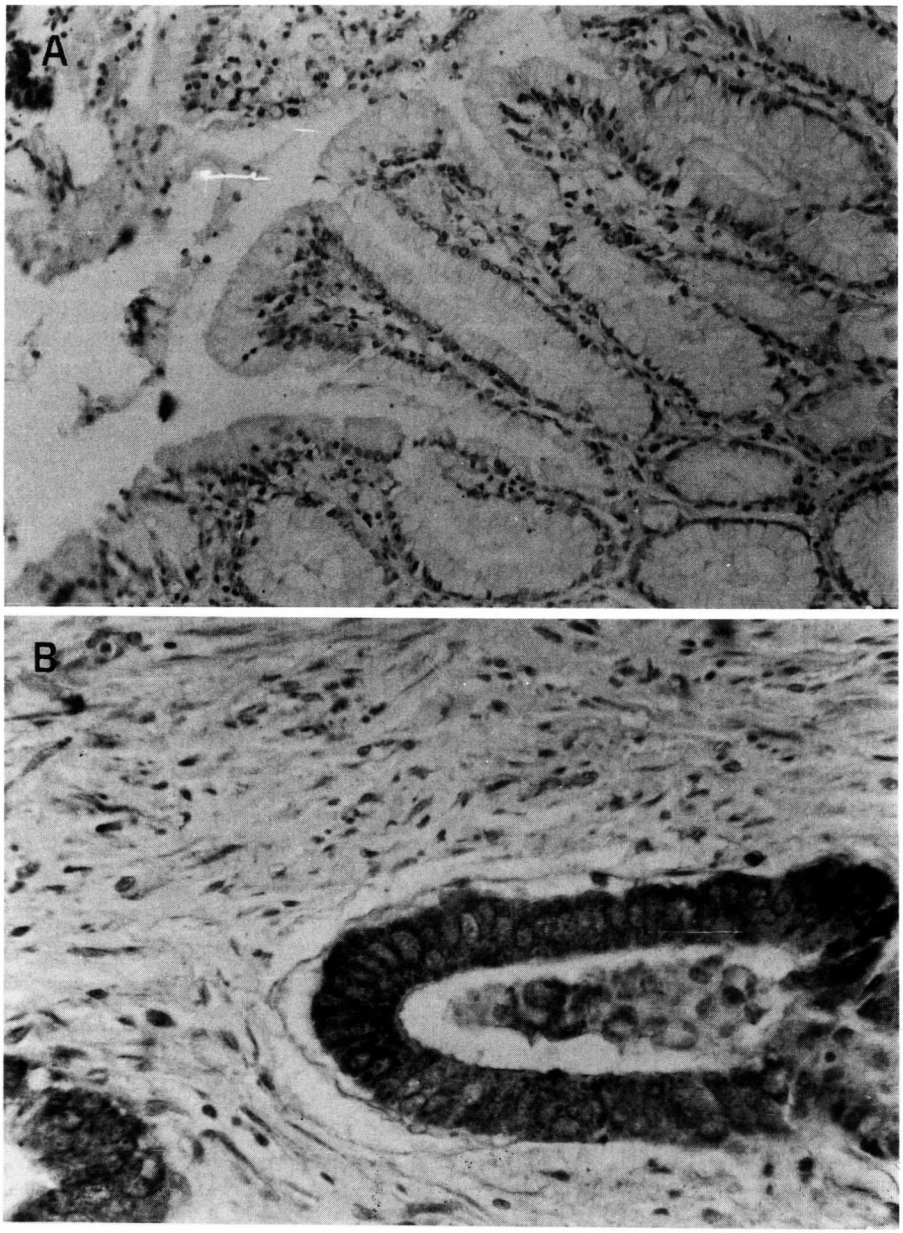

Fig. 2 Immunohistochemical staining of stomach tissues with the rp28 antibody. A, a normal gastric mucosa not reactive to rp28 (200×). B, a well differentiated tubular adenocarcinoma with strongly positive cytoplasmic staining (400×).

Our immunohistochemical results uncovered a relationship between the degree of cancer differentiation or histological cell types and the level of *ras* p21 expression. In contrast to Ohuchi *et al.*'s findings (5), cells of well and moderately differentiated tubular adenocarcinomas and papillary adenocarcinomas contained larger amounts of Ha- and/or Ki-*ras* p21 than those of poorly-differentiated and mucinous adenocarcinomas and undifferentiated carcinomas. Although differential detection of c-Ha-*ras*, c-Ki-*ras* and N-*ras* in gastric lesions remains to be analyzed, our present results suggest that *ras* p21 expression might be important for the maintenance of the glandular structure in tubular adenocarcinomas of the stomach.

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