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An Interaction Between ras-p21 and nm23 Influencing the Clinical Course of Gastric Carcinoma

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Summary. **Combined expressions of ras-p21 and nm23 and their correlation with clinicopathological factors were examined in patients with gastric carcinoma. These expressions were determined by immunohistochemical analysis of 60 formalin-fixed and paraffin-embedded specimens of carcinoma that were curatively resected. Distribution of ras-p21 and nm23 expressions differed according to the macroscopic type of tumor, and the mode of these expressions was prognostically related: nm23 expression singly affected the survival activity, and a combined expression of ras-p21 and nm23 induced a very short period of survival.**

These findings suggest that there is an interaction between ras-p21 and nm23, and that analysis of the mode of the combination is useful in predicting the clinical course of gastric carcinoma.

Key words—digestive tract, malignant tumor, immunohistochemical analysis, gene expression.

INTRODUCTION

ras-p21 and nm23 composed of a complex of GTP-binding proteins are involved in the pathways which control cell cycle progression, and they have been recognized as tumor modifiers.[1)] In connection with this, ras-p21 and nm23 have been shown to be reciprocally active in the metastatic potential of specific kinds of carcinoma.[2-8)] In gastric carcinoma, the relation of these expressions to carcinogenesis has been explored, and their clinicopathological features have also been characterized,[9-13)] but the effect of the combined mode of these expressions on the clinical course after surgery has not yet been examined.

This study was designed to investigate whether analyzing combined expressions of ras-p21 and nm23 has clinicopathological significance in patients with gastric carcinoma.

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MATERIALS AND METHODS

Patients

Surgical specimens were obtained from 60 patients with gastric carcinoma (Table 1). The age of the patients ranged from 30 to 70 years, and the average was 52.0±1.0 (mean±SEM) years. They had received curative surgery including resection at The Abdominal Surgery, Third Hospital, Harbin Medical University, between 1982 and 1985. Histological evaluation was based on routine examination of paraffin embedded sections stained with hematoxylin and eosin. The histological type was classified according to the general rules for gastric cancer.[14)] The clinical staging (TNM system) was determined according to the guidelines of the American Joint Committee on Cancer.[15)]

Immunohistochemical staining

All the specimens containing normal tissue adjacent to the tumor tissue were stained with antibodies to ras-p21 and nm23/NDPK A, according to the streptoavidin-biotin complex method. A mouse monoclonal antibody (MoAb) , RAP-5 (IgG2a isotype, Oncogene Science, Inc., NY) , and anti-human nm23 antibody (Transduction Laboratories, KY) were used. Four-μm sections prepared on glass slides were dewaxed. In order to restore the immunoreactivity of the

Table 1. Clinical and histological variations in 60 patients with gastric carcinoma

Variables	Number of patients (%)
Sex	
Male	37 (62%)
Female	23 (38%)
Age (years)	
30-45	13 (21%)
46-60	37 (62%)
>61	10 (17%)
Tumor site	
U	2 (3%)
M	1 (2%)
ML	3 (5%)
LM	15 (25%)
L	36 (60%)
MLU	3 (5%)
Tumor stage	
I	11 (18%)
II	22 (37%)
III	13 (22%)
IV	14 (23%)

antigens, the specimens were treated in 10 mM citric acid (pH 6.0) in a microwave (100V, 800W) for 10 min, and then cooled to room temperature in phosphate buffer saline (PBS). Endogenous peroxidase activity and nonspecific binding were blocked by treatment with 3% hydrogen peroxidase for 50 min and with 10% normal rabbit serum for 10 min. Specimens were incubated with anti-ras-p21 MoAb at room temperature for 60 min. After rinsing in PBS, the specimens were incubated with peroxidase-labeled streptoavidin-biotin for 20 min at room temperature, and further treated with 0.05% 3,3'-diaminobenzidine solution for 5 min at room temperature. After washing in distilled water, they were counter-stained with hematoxylin and eosin.[16] Negative controls were obtained by omission of the primary antibody during incubation. The tumer tissue was stained evenly, and the staining of cells in lymph node metastases was the same as in the primary tumor.

Judgement for staining

Staining of ras-p21 and nm23 was evaluated on the basis of the percentage of tumor cells showing signs of cytoplasmic reactivity, and the results were classified as follows: For ras-p21: 1) negative (-), 0% tumor cells showing positive reactivity; 2) partly positive (+), 1-10% of tumor cells showing positive reactivity; and 3) moderately positive (++, overexpression), >10% of tumor cells showing positive reactivity. For nm23: 1) negative (-), 0% tumor cells showing positive reactivity; 2) partly positive (+), 1-20% of tumor cells showing positive reactivity; and 3) diffusely positive (++, overexpression), >20% of tumor cells showing positive reactivity.

Statistical analysis

Associations between the expression of ras-p21 and nm23 and clinicopathologic factors were analyzed by means of the X^2 test. The correlation between ras-p21 and nm23 was estimated by regression analysis. Kaplan-Meier's and Logrank analyses were performed to assess whether ras-p21 or nm23 expression, alone or in combination, had any effect on the survival period. The statistical difference was considered significant if the p value was <0.05.

RESULTS

ras-21 and nm23 expression

When ras-p21 expression in 60 patients was analyzed according to our judgement for staining (Fig. 1), the frequency of ras-p21−, ras-p21+ and ras-p21++ was 11, 10, and 39 cases, respectively. This expression was not distributed evenly ($p<0.01$). In the case of nm23 expression (Fig. 1), the frequency of nm23−, nm23+ and nm23++ was 11, 8, and 41 cases, respectively. This distribution was not even ($p<0.01$). No significant relationship existed between ras-p21 and nm23 expression ($r=0.1350$, $p>0.05$).

ras-p21 and nm23 expression and pathological factors

When the relation of ras-p21 and nm23 expression to pathological variables was analyzed, the distribution of ras-p21 or nm23 expression differed according to the histological grade and macroscopic type of tumor (Table 2 and 3). Lymph node metastasis did not correlate with the mode of ras-p21 and nm23 expression (Table 4). Neither tumor site nor tumor stage correlated with ras-p21 and nm23 expression, alone or in combination (data not shown).

ras-p21 and nm23 expression and survival

Kaplan-Meier's survival curves and Logrank analyses demonstrated that there was a significant difference among −, + and ++ patients showing nm23: cases with + expression had a shorter overall survival than those with − and ++ expression (Fig. 2). No significant difference in survival period was detected in patients showing ras-p21. When ras-p21 and nm23 expressions were combined, it was noted

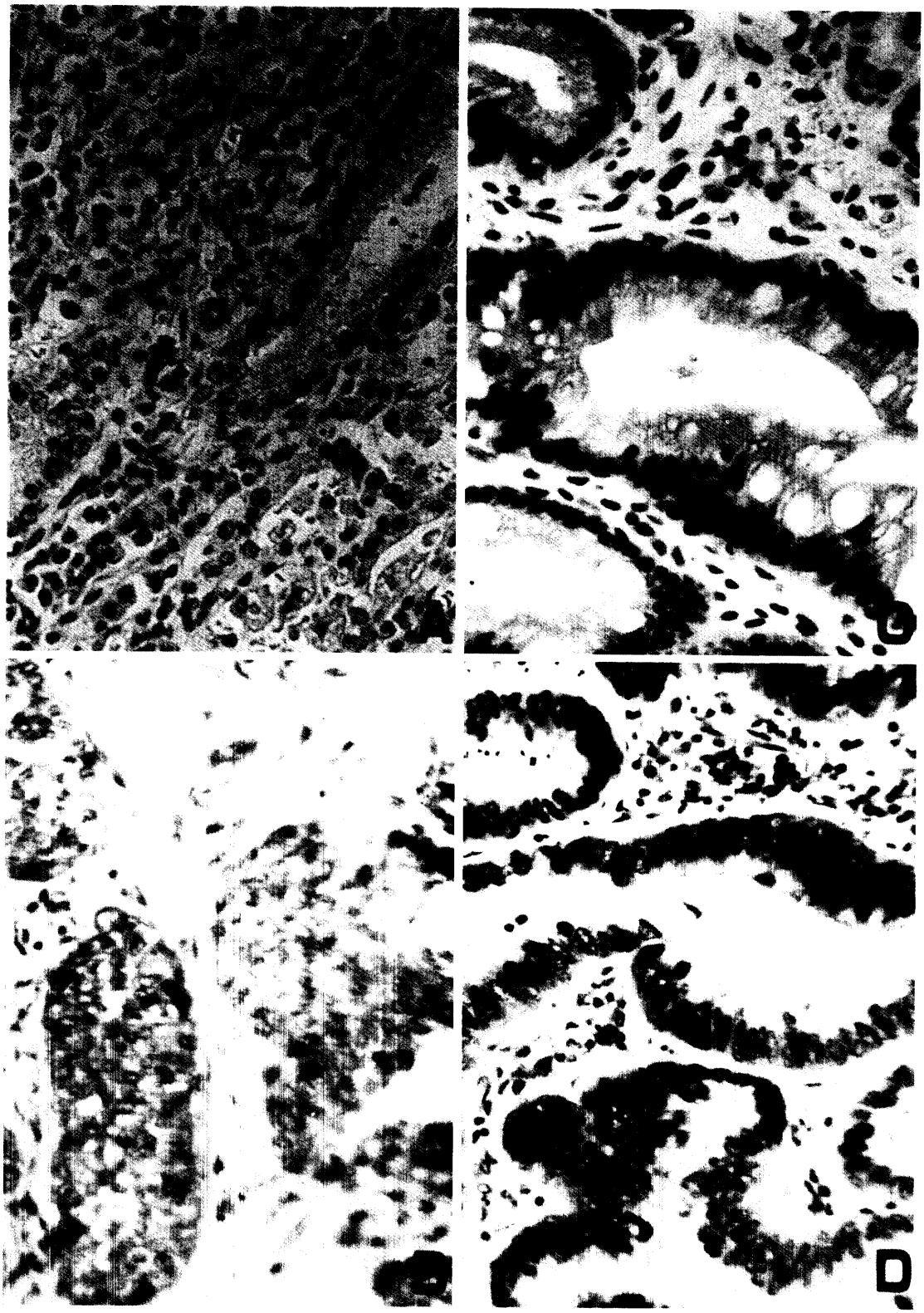

Fig. 1. Immunostaining of cytoplasmic nm23 and ras-p21. **(A)** Negative (−) and **(B)** partly positive (+) of nm23. **(C)** Negative (−) and **(D)** moderately positive reactivity of ras-p21 (++). ×400

Table 2. Distribution of ras-p21 and nm23 expression in tumor grade

Histological type	ras-p21 staining			nm23 staining			Total
	−	+	++	−	+	++	
Papillary	1	2	0	0	3	0	3
Tubular	3	6	10	3	2	14	19
Poorly	3	2	26	8	3	20	31
Signet ring	1	0	2	0	1	2	3
Mucinous	1	1	2	1	1	2	4
p-value	<0.05			>0.05			

Table 3. Distribution of ras-p21 and nm23 expression in macroscopic type

Type	ras-p21 staining			nm23 staining			Total
	−	+	++	−	+	++	
I	2	3	1	2	0	4	6
II	3	4	23	6	3	21	30
III	3	3	16	3	3	16	22
IV	1	1	0	0	2	0	2
p-value	<0.05			<0.05			

Table 4. Lymph node metastasis and ras-p21 and nm23 expression

Grade	ras-p21 staining			nm23 staining			Total
	−	+	++	−	+	++	
N 0	2	2	11	1	2	12	15
N 1	5	4	15	7	2	15	24
N 2	1	0	6	0	0	7	7
N 3	3	4	7	3	4	7	14
p-value	>0.05			>0.05			

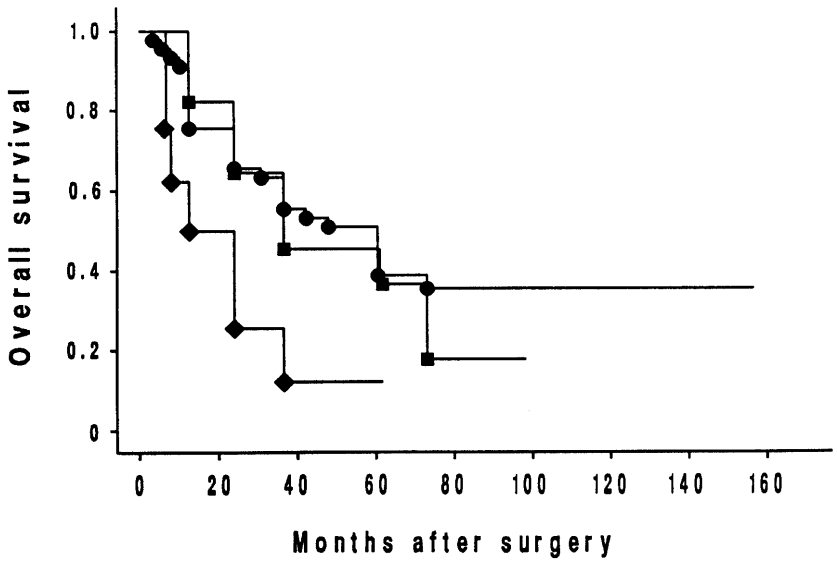

Fig. 2. Overall survival and nm23 staining in patients with gastric carcinoma. The difference between nm23+ (◆, n=8) and nm23++ (●, n=41) groups was significant ($p<0.01$). The difference between nm23+ (◆, n=8) and nm23− (■, n=11) groups was significant ($p<0.05$).

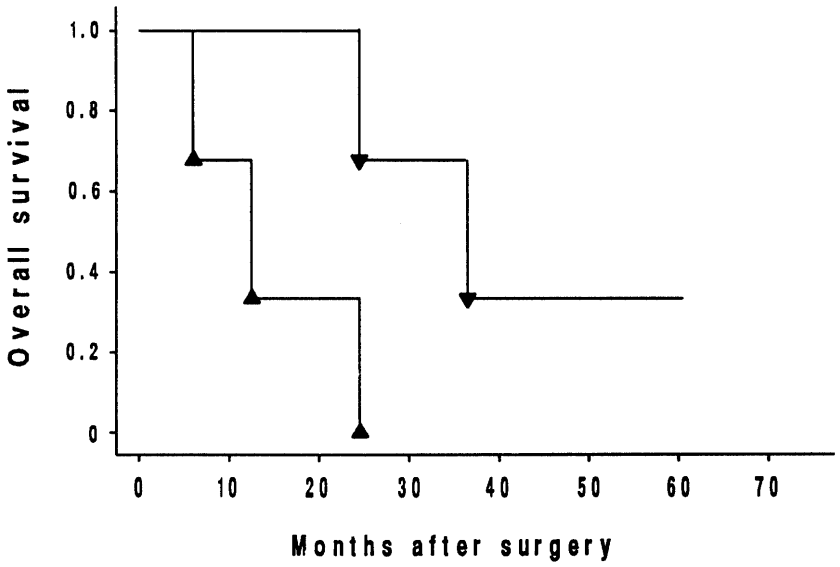

Fig. 3. Overall survival and ras-21 and nm23 staining in patients with gastric carcinoma. The difference between nm23+/ras-p21++ (▲, n=3) and nm23+/ras-p21+ (▼, n=4) groups was significant ($p<0.05$).

that patients with ras-p21++ and nm23+ expression had the shortest survival period (Fig. 3).

DISCUSSION

We found that ras-p21 interacts with nm23 in expressing clinicopathological features of gastric carcinoma. This is partially consistent with a report that genes interact with each other in expressing the features of several kinds of carcinoma.[17-20]

Although conflicting data have been presented,[21-23] evidence of the metastatic suppressor function of nm23[24,25] has been reported, and overexpressed nm23 has been shown to indicate a favorable prognosis in several types of carcinoma.[8,26] Contrary to this, ras-p21 has been shown to indicate a negative prognosis in carcinoma.[19,27] In this study, although the metastasis was not small in patients with nm23 overexpression, the survival activity was strong, and the activity was reduced by ras-p21 overexpression even when nm23 was positively expressed. This could mean that excessive nm23 contributes to the enhancement of the survival activity, but ras-p21 dominantly interacting with nm23 impedes the activity.

Biochemically, ras-p21 and nm23 have been shown to form a complex with GTP-binding proteins,[1] and to cause a reduction in cell migration in response to insulin-like growth factor I[24] and platelet-derived growth factor as well as altered colonization in response to transforming growth factor-beta.[25] Of interest is the fact that ras-p21 and nm23, having a similar structure, reciprocally affect cancer cell growth and its aggressiveness through the factors mentioned above.

Negative nm23 cases have been shown to induce high amounts of ras-p21, thus providing a biochemical basis to explain the negative prognosis.[8] In fact, it has been reported that the overexpression of ras-p21 is associated with the metastatic phenocyte *in vitro*[3] and with a negative prognosis in ovarian cancer.[2] In this immunohistochemical analysis, no inverse relationship was detected between the two, and ras-p21 seemed to be the determinant. There is much room for research into this aspect.

Invasive behavior of a tumor is determined by expansive components.[14] In this study, the histological type and macroscopic pattern of the tumor were attributed to the distribution of ras-p21 and nm23 expression. Considering this together with the result showing that the stage of the tumor did not relate to these expressions, it is possible that these expressions partly contribute to the invasive behavior of the carcinoma.

The increased number of regional lymph node metastases has been shown to be correlated with a poor prognosis for gastric carcinoma.[28,29] In this analysis, no relation of metastasis to ras-p21 and

nm23 was detected, but the prognosis was dependent on these expressions. ras-p21 and nm23 might be markers independent of metastasis.

These observations have led us to conclude that an analysis of the interaction between ras-p21 and nm23 is useful in evaluating the clinical course of gastric carcinoma.

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