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Clinicopathological Role of Nm23 Expression in Early Gastric Cancer

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Relations of nm23 expression to 8 clinicopathologic variables and proliferative activity of cancer cells were examined in 22 patients with early gastric cancer, to clarify the role of nm23 in the cancer lesions. The expression in cancer lesions was immunohistochemically analyzed, and the proliferative activity was evaluated by proliferating cell nuclear antigen (PCNA) labeling index (LI). Expression of nm23 was found in 11 lesions (positive group) but not in 11 lesions (negative group). A significant ($P < 0.05$) difference between the positive and negative groups was found in only one, histologic type, out of the clinicopathologic variables: differentiated adenocarcinoma was more in the positive group than in the negative group. PCNA LI ($46.79 \pm 10.27\%$) in the former was significantly ($P < 0.01$) higher than that ($30.79 \pm 11.15\%$) in the latter. These results suggest that expression of nm23 is closely related to the tumor differentiation and proliferation of cancer cells in early gastric cancer but not to lymphnode metastasis or lymphatic invasion.

Key Words

Early gastric carcinoma,
Nm23 expression,
Proliferating cell nuclear antigen (PCNA).

INTRODUCTION

Nm23 has been well-known as one of the metastasis suppressor genes, and the expression has been reported to be related to the good prognosis and a lack of lymphnode metastasis in breast carcinoma.[1-4] However, the conflicting results have been also reported in various malignant tumors.[5-7] Thus, the role of nm23 expression is controversial in malignant tumor. Furthermore, few studies on the significance of nm23 expression have been performed in gastric carcinoma.[8,9]

In the current study, expression of nm23 in early gastric cancer was examined by studying the relations of the expression to the clinicopathology of the cancer lesions and/or patients and to the proliferative activity of cancer cells, which have been already confirmed as prognosticators in gastrointestinal cancer,[10,11] to clarify the role of nm23 in the cancer lesions.

MATERIALS AND METHODS

1. Patients and clinicopathologic examination

Twenty-two early gastric cancer patients who underwent gastrectomy in the First Department of Surgery from 1993 to 1994 were included in this study. Resected specimens were

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fixed in 4% buffered formalin and paraffin-embedded. To avoid the reduction of immunoreactivity, the time of fixation did not exceed 48 hours. Clinicopathologic examinations were carried out routinely with hematoxylin-eosin stained slides, and the paraffin-embedded blocks containing the central sections of the cancer lesions were selected in each case. Eight clinicopathologic variables consisting of age, size, location, gross type, histologic type, depth of invasion, lymphatic invasion and lymphnode metastasis were examined according to the "Japanese Classification of Gastric Carcinoma".[11] However, histologic type in the current study was classified into 2 major groups: differentiated and undifferentiated adenocarcinomas. The former included papillary and tubular adenocarcinomas, and the latter consisted of poorly differentiated adenocarcinoma and signet-ring cell carcinoma.

2. Immunohistochemical analysis of nm23

Four μm sections in parallel with a central section of cancer lesions were made from the paraffin-embedded cancer tissues. Sections were dewaxed in 100% xylene and dehydrated through graded alcohols. Endogenous peroxidase activity was blocked by pre-incubation with 3% hydrogen peroxide. Nonspecific bindings were also blocked with normal rabbit serum. Then, the sections were incubated with a primary antibody, mouse monoclonal anti-human nm23-H2-1 antibody (H2-206, 2μg/ml, Seikagaku Co., Tokyo, Japan), at 4 ℃ overnight. Thereafter, the sections were washed with phosphate buffered saline (PBS),

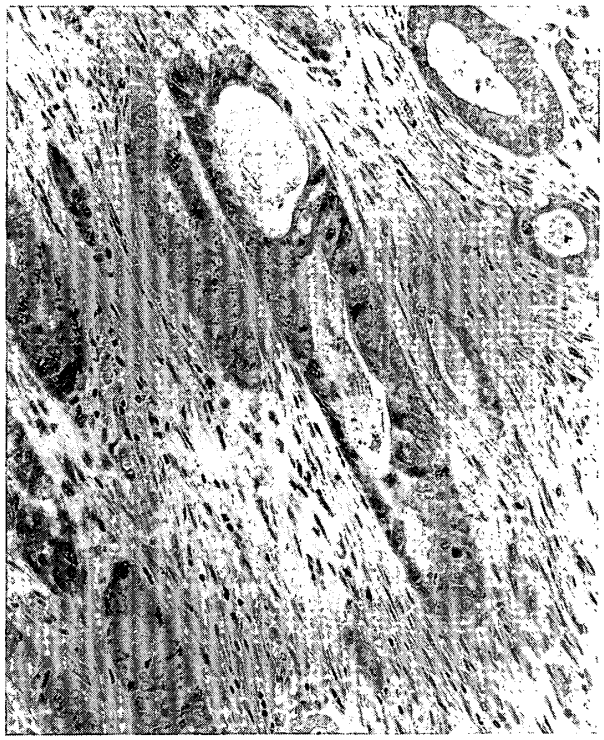

Figure 1. Immunohistochemical stain of nm23. Most of all the cancer cytoplasms were brownish stained, but the interstitial tissues and/or non-cancer cells were not stained. These cancer lesions were treated as "positive". Original magnification ×200.

and incubated with biotinylated rabbit anti-mouse immunoglobulin G (10 μg/ml) at room temperature for 10 minutes. After washing with PBS, they were incubated with streptoavidin-biotin-peroxidase complex (100 μg/ml, Nichirei Co., Tokyo, Japan). As a chromogen, 3-3'-diaminobenzene tetrahydrochroride was used with hydrogen peroxide in Tris buffer. The sections were counterstained with hematoxylin. The grade of the immunoreactivity was examined by 2 of us (T. N. and Y. T.) without knowledge of the clinical details, and the lesions were classified into 2 groups by the immunoreactivity of nm23: negative group with a little

Table 1. Relationship between expression of nm23 and 8 clinicopathologic variables in early gastric cancer.

Clinicopathologic variables		Expression of nm23	
		Positive (n=11)	Negative (n=11)
age (mean ± SD)		70.91 ± 7.05	65.09 ± 9.47
size (mean ± SD)		2.83 ± 0.91	3.34 ± 1.04
location	C	1	2
	M	5	7
	A	5	2
gross type	protruded	2	1
	excavated	9	10
histological type †	dif	11	6
	undif	0	5※
depth of invasion	mucosa	3	6
	submucosa	8	5
lymphatic invasion	negative	4	7
	positive	7	4
lymphnode metstasis	negative	8	10
	positive	3	1

† Dif and undif indicate differentiated and undifferentiated adenocarcinomas.

※ Significant (P<0.02) difference between both groups.

or no reactivity and positive group with strong reactivity (Fig. 1).

3. Immunohistochemical analysis of proliferating cell nuclear antigen (PCNA)

To evaluate the proliferative activity of cancer lesions and/or cells, immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) was performed in the almost same procedures as the aforementioned nm23 immunohistochemical staining methods. Nonspecific bindings were blocked with normal bovine serum. As a primary antibody, PC10 (Dakopatts, Glostrup, Denmark) was used at a 1:20 dilution at 4 ℃ overnight. As a chromogen, 3-amino-9-ethylcarbazol was used. One thousand of cancer cells were examined and PCNA labeling index (LI) was expressed as the ratio (%) of PCNA positive cancer nuclei to all of the examined nuclei, as already reported by us.[10]

4. Statistical Analysis

The data were analyzed by the chi-square or Fisher's exact probability calculation tests and Student's *t* test. P values less than 0.05 were taken to be significant.

Table 2. Relationship between expression of nm23 and proliferative activity in early gastric cancer.

Expression of nm23	PCNA LI (mean ± SD)
positive (n=11)	46.79 ± 10.27 ※
negative (n=11)	30.79 ± 11.15

※ Significant (P<0.01) from the negative group.

RESULTS

1. Relationship between nm23 expression and clinicopathologic variables

Expression of nm23 was found in 11 lesions (positive group) but not in 11 lesions (negative group). The relations of nm23 expression to 8 clinicopathologic variables were examined in these 2 groups. As shown in Table 1, a significant relationship between nm23 expression and histologic type was found: differentiated adenocarcinoma in the positive group was significantly ($P < 0.02$) more than in the negative group, and undifferentiated adenocarcinoma was not found in the positive group. However, the other variables showed no significant difference between 2 groups (Table 1).

2. Relationship between nm23 expression and proliferative activity

The PCNA LI (mean ± standard deviation) was 46.79 ± 10.27% in the positive group and 30.79 ± 11.15% in the negative group. The PCNA LI in the former was significantly ($P < 0.01$) higher than that in the latter (Table 2).

DISCUSSION

Although reduced expression of nm23 gene has been reported to correlate with metastatic potential in various carcinomas,[1-4,8,9,12-14,16] the opposite results have been also reported in various malignant tumors.[5-7] Moreover, the role of nm23 expression in gastric cancer has not yet been fully examined. Thus, the expression of nm23 was immunohistochemically examined in early gastric cancer, and its relations to clinicopathology and proliferative activity of cancer cells, which have been already confirmed as the prognostic indicators,[10,11] were analyzed in this study.

Clinicopathologically, a significant relationship was found only one, histologic type, out of 8 clinicopathologic variables. Namely, differentiated adenocarcinoma in the positive group was significantly more than in the negative group. However, no significant difference was found in any of the other variables: significant relations of nm23 expression to lymphnode metastasis and/or lymphatic invasion was not shown in the current study. The nm23 gene product has been reported to be identical with a nucleoside diphosphate (NDP) kinase.[18] Furthermore, the expression of nm23 has been reported to be closely related to the lack of lymphnode metastasis in breast carcinoma.[1-4] Thus, the result in the current study suggests that nm23 expression in early gastric cancer lesions is closely related to tumor differentiation but not to lymphnode metastasis and/or lymphatic invasion.

In this study, a significant difference between the positive and negative groups was found in the proliferative activity represented by PCNA LI: the LI in the positive group was significantly higher than that in the negative group. Concerning the relationship between proliferative activity and nm23 expression, the correlation has been also reported in acute leukemia[22] and prostate carcinoma.[23] NDP kinase, nm23 gene product, has an important role in signal transduction supplying GTP to GTP-binding protein.[21] Tublin, which is thought to be one of the GTP-binding proteins,[19,20] plays an important role in mitosis. Thus, the result seems to indicate that expression of nm23 in the cancer lesions is closely related to the proliferative activity of cancer cells represented by PCNA LI.

From the aforementioned results and facts, it may be concluded that expression of nm23 in early gastric cancer lesions is related to the tumor differentiation and/or proliferation but not to the lymphnode metastasis or lymphatic invasion.

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