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Reduced Expression of nm23 Protein is Related to Nodal Metastasis of Human Gastric Carcinoma

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= Abstract = **Reduced expression of nm23 gene or protein has been known to be related with nodal metastasis in a variety of malignant tumors of the breast, lung, liver, prostate, ovary and stomach. To elucidate a possible prognostic factor, we studied 42 cases of gastric adenocarcinomas for the expression of nm23 protein using immunohistochemical methods and compared with the known prognostic parameters. The nm23 protein was intensely stained in the cytoplasm and/or the nucleus of carcinoma cells in 9 cases(21.4%). The nm23 protein expression of the non-metastatic group(46.7%) was higher than that of the nodal metastasis group(7.4%). Perigastric lymph node metastases($p<0.001$) were more frequently found in the nm23 protein negative group(75.8%) than in the nm23 positive group(22.2%). There was no significant correlation between nm23 protein expression and other parameters such as patient age, sex, WHO grade, Lauren classification, depth of invasion, location of tumor and size. The results suggest that nm23 protein expression plays a role in suppression of nodal metastasis in the gastric adenocarcinoma.**

Key words: *nm23, Nucleoside diphosphate Kinase, Stomach, Cancer, Mta- stasi*

INTRODUCTION

The nm23 gene has been reported to be a metastasis suppressor gene based on the experiment using murine K-1735 melanoma cell lines with low and high metastatic potential (Steeg *et al.* 1988a&b). The transfection of nm23 gene into a highly metastatic murine melanoma cell line resulted in the reduction of metastatic potential and primary tumor formation without differences in intrinsic tumor cell growth (Leone *et al.* 1991). The gene is located in the long arm of chromosome 17 (Varesco *et al.* 1992; Stephenson *et al.* 1993) and is known to be related with nucleoside diphosphate (NDP) kinase (Kimura *et al.* 1988&1990; Liotta & Steeg 1990; Gilles *et al.* 1991). Negative correlations of nm23 expression with human cancers from the breast (Bevilacqua *et al.* 1989; Barnes *et al.* 1991; Hirayama *et al.* 1991; Royds *et al.* 1993; Yamashita *et al.* 1993; Simpson *et al.* 1993), ovary (Foulkes *et al.* 1993; Mandai *et al.* 1994), prostate (Konishi *et al.* 1993; Brewster *et al.* 1994; Igawa *et al.* 1994), pancreas (Nakamori *et al.* 1993), colon and rectum (Leone *et al.* 1991; Cohn *et al.* 1991; Myeroff *et al.* 1993; Yamaguchi *et al.* 1993; Bafico *et al.* 1993), lung (Leone *et al.* 1991; Engel *et al.* 1993), thyroid (Arai *et al.* 1993; Zou *et*

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al. 1993; Farley *et al.* 1993) and stomach (Nakayama *et al.* 1993; Kodera *et al.* 1994) have been reported.

Because gastric cancer still remains the leading cause of cancer death in Korea, it would be of great significance if something other than the known clinical or histopathologic parameters could be predictable for the metastasis and prognosis of gastric cancer. We studied the nm23 protein expression of surgically resected gastric cancer tissues, with the hope that the immunohistochemical expression of nm23 protein might be one of such predictive factors.

MATERIAL AND METHOD

1. Material

Tissues were obtained from 42 surgically resected stomachs in the Department of Surgery, Seoul National University Hospital during the period from January 1990 to December 1992, with age and sex matching.

2. Method

Samples were fixed with 10% neutrally buffered formalin for 24 hours, processed routinely, and embedded in paraffin. Paraffin-embedded tissues were cut into 5 um-thick sections and processed immunohistochemically. The tissues were deparaffinized, treated by 0.3% H2O2 for 15 minutes and blocking serum for 30 minutes. Then, we used primary antibody, NCL-nm23 (Novocastra Laboratories Ltd.), with the dilution of 1:200 at room temperature for 2 hours. Consequently, avidin-biotin peroxidase complex method was performed using an LSAB kit (DAKO). Finally, the tissues were stained with diaminobenzidine and were counter-stained with hematoxylin.

For the statistical analysis, we used Fisher's exact test or Chi-square test using the PC-SAS program, 6. 04 version.

RESULTS

The nm23 protein was intensely stained in the cytoplasm and/or the nucleus of carcinoma cells in 9 (21. 4%) out of 42 cases (Fig. 1). Although some cases showed weak staining, we discarded the cases that showed stainability either weaker than the adjacent non-cancerous mucosa or heterogeneous staining. Some cases showed more intense staining in the well differentiated area and weaker staining in the poorly differentiated area. Furthermore, the deeper portion of the tumor showed the weaker staining in cases. The nm23 protein expression was definite in 7 of 15 cases (46.7%) of the non-metastatic group, while it was definite in 2 of 27 cases (7.4%) of the nodal metastasis group (Fig. 2). Thus the positive ratio was much higher in the non-metastatic group, and it was statistically significant by a p value less than 0.01. We tried to compare this finding to other data related to the prognosis. There was no significant correlation between nm23 protein expression and other parameters such as patient age, sex, WHO grade, Lauren classification, depth of invasion, location of tumor and size (Table 1) although the Lauren's diffuse type and larger tumor (>5 cm) were more frequently found in the nm23 negative group than in the nm23 positive group (Fig. 3). Only perigastric lymph node metasis was significantly associated with nm23 protein expression ($p<0.001$).

DISCUSSION

The actual mechanism by which nm23 gene or product affects invasive or metastatic potential still remains unknown. Because of the homology between the nucleoside diphosphate (NDP) kinase and the role of NDP kinase in cytoskeleton status and cell to cell communication (Nickerson & Wells 1984; Gilles *et al.* 1991), it could be predicted that the nm23 takes part in cell adhesion or migration, namely invasion or metastasis (Kodera *et al.* 1994). However, controversies are still present, and additional studies are mandatory.

The expression of nm23 gene has been known to be inversely correlated with metastatic potential in various human cancers as well as gastric cancer. In gastric cancer, it has been reported that nm23 gene expression was higher in primary cancer tissue than in matched mucosa (Nakayama *et al.* 1993). Furthermore, it has been suggested that

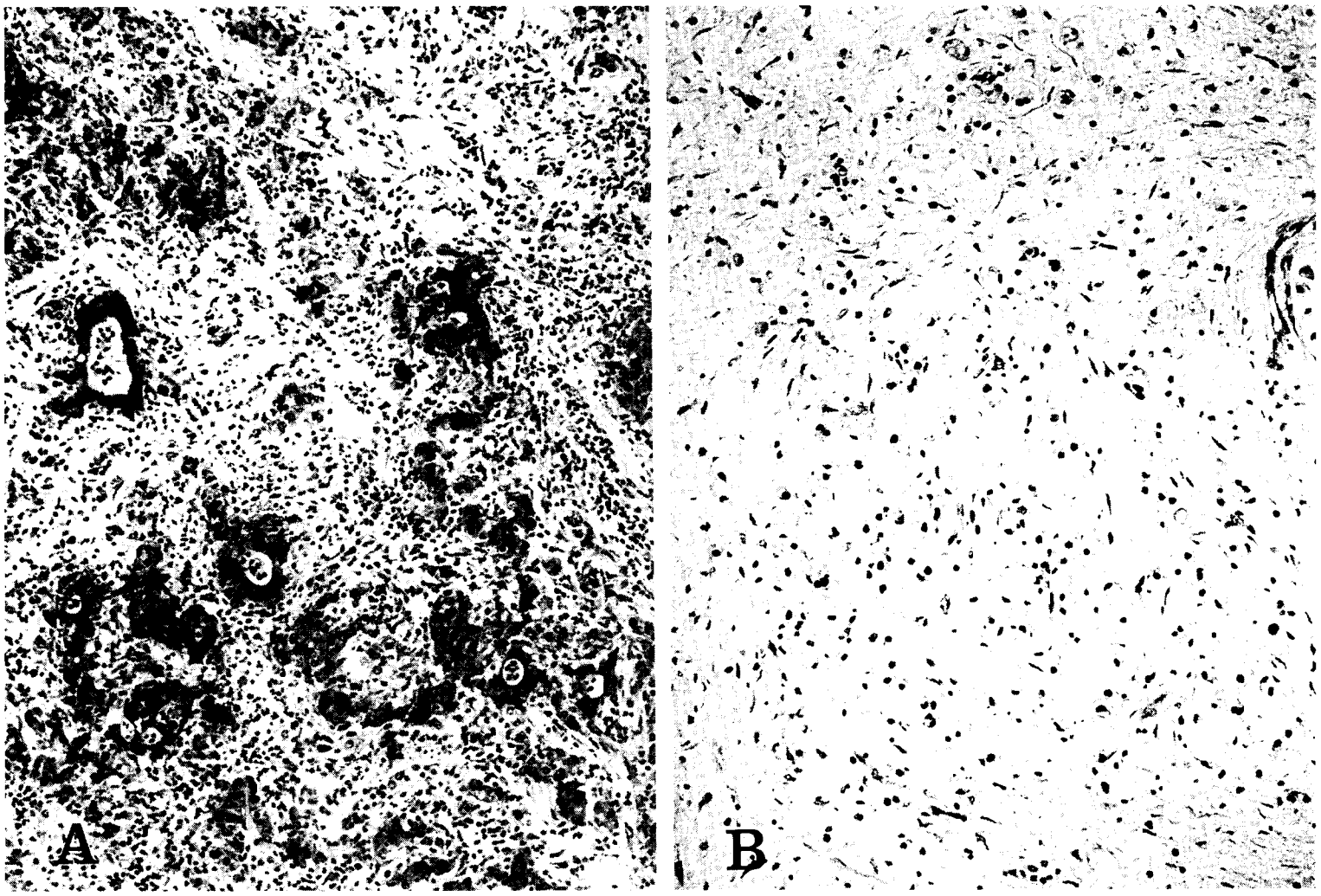
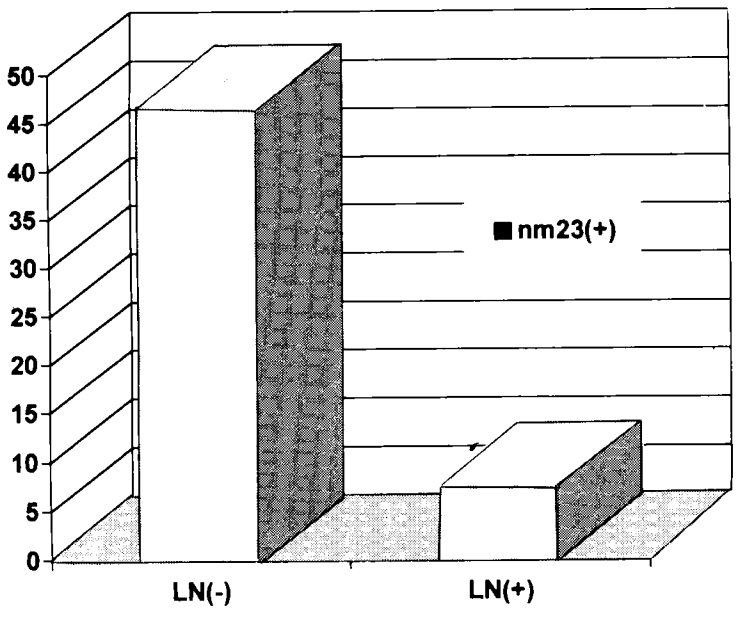


Fig. 1. Photomicrographs of the nm23 positive (A) and negative (B) gastric cancer cells



. The nm23 protein expression rate according to lymph node metastasis

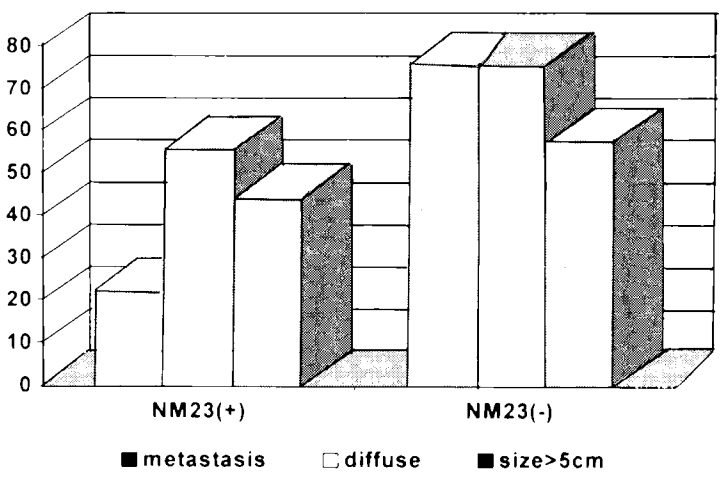


Fig. 3. The rates of lymph node metastasis, Lauren's diffuse type and large tumor size (>5 cm) according to nm23 protein expression

Table 1. Relationship between nm23 protein expression and clinicopathologic parameters in gastric adenocarcinoma

Parameters	nm23 reactivity		p value
	negative	positive	
Samples(n = 42)			
Nodal metastasis			<0.001
absent	8(53.3)	7(46.7)	
present	25(92.6)	2(7.4)	
Tumor size			NS
5 cm <	14(73.7)	5(26.3)	
5 cm >	19(82.6)	4(17.4)	
Lauren classification			NS
intestinal	8(66.7)	4(33.3)	
diffuse	25(83.3)	5(16.7)	
Sex			NS
male	25(78.1)	7(21.9)	
female	8(80.0)	2(20.0)	
T classification			NS
T2	12(80.0)	3(20.0)	
T3	21(77.8)	6(22.2)	
WHO grade			NS
well	1(50.0)	1(50.0)	
moderately	8(72.7)	3(27.3)	
poorly	24(82.8)	5(17.2)	

NS: not significant

the down-regulation of nm23 gene might have a role in metastasis and invasion in gastric cancer, possibly leading to a poor prognosis (Kodera *et al.* 1994). With regard to the loss of heterozygosity, it was not only rare or hardly found but was also not related with clinico-pathologic features (Nakayama *et al.* 1993; Kim *et al.* 1993). The current study demonstrates that reduced expression of nm23 protein in gastric adenocarcinoma would be related with lymph node metastasis. Some factors, such as Lauren's diffuse type and large tumor size, were also related with the down-regulation of nm23 protein expression although there was no statistical significance. There has been controversy as to whether the differentiation of gastric adenocarcinoma is associated with nm23 expression (Kodera *et al.* 1993; Nakayama *et al.* 1994). The action mechanism of nm23 in cancer might be explained by a bimodal pattern. First, it might act as a suppressor of invasion in mammary ductal carcinoma in situ (Simpson *et al.* 1994). Second, it might act as a well-known suppressor of lymph node metastasis. Gastric carcinogenesis has been explained by two pathways, de novo or by way of colonic metaplasia. In that sense, we presume that the genetic events of gastric carcinomas with reference to Lauren's classification would show discrepancy with each other. Our study has the limitations of a small number of cases and short follow-up period. Therefore, we are preparing more reliable data with a larger number of cases with longer follow-up period.

In Korea, studies related to the metastatic potential of human cancer and nm23 expression have only been performed in breast carcinomas, especially using the immunohistochemical method

(Song *et al.* submitted). Although the loss of heterozygosity has been studied in gastric adenocarcinoma, the result was not contributory (Kim *et al.* 1993). We hope that additional extensive studies elucidating the role of nm23 in the metastasis of various human cancers will be performed in the near future.

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