Original Articles

The Significance of nm23 Protein Expression in Human Gastric Carcinomas

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Abstract: The clinical significance of nm23 protein (nm23) expression was studied in tissue samples from 110 patients with primary gastric cancer by immunohistochemical staining with the anti-nm23 antibody. Primary carcinomas with either lymph node involvement or liver metastasis expressed significantly reduced levels of nm23 compared to those without metastasis. This relationship was clearer in the more differentiated adenocarcinomas than in the poorly differentiated adenocarcinomas. However, there was no correlation between nm23 expression and depth of invasion, quantity of stroma, infiltrating growth pattern, or macroscopic type. The cumulative 5-year survival rates based on nm23 immunoreactivity within the primary tumor were significantly higher in the nonreduced expression group (72%) than in the reduced expression group (45%). A multivariate analysis revealed that nm23 expression levels influence the outcome of patients as strongly as depth of invasion and more strongly than the other clinicopathological factors. These results suggest that the degree of nm23 expression is closely related to the metastatic potential of gastric carcinoma cells and can be used as a prognostic indicator independent of the clinicopathological features.

Key Words: gastric cancer, nm23, metastasis of gastric cancer

Introduction

Tumor metastasis is the main cause of treatment failure in cancer patients. Therefore, it is crucial to identify its presence so that the most appropriate therapy can be selected and the patient’s prognosis can be predicted. These predictions have typically been based on clinicopathological findings alone; however, recent remarkable developments in molecular biology have provided valuable techniques of assessing malignant potential more accurately. The nm23 gene in particular has attracted attention as being related to metastasis, following the results of some experiments.1,2 This gene was originally identified by differential screening of a murine K1735 melanoma cell line cDNA library using mRNA derived from cell lines with differing metastatic potential. Expression of the nm23 gene was found to be inversely related to metastatic potential, with tenfold higher mRNA levels in cell clones with low metastatic potential than in those with high metastatic potential.1 Therefore, it is thought that nm23 protein (nm23) may play a specific biological role in suppressing tumor metastasis. In fact, the suppression of metastatic potential was demonstrated experimentally by transfecting nm23 cDNA into high-metastatic tumor cells.2 It has also been reported that breast cancer with reduced expression of the nm23 gene was associated with more frequent lymph node metastasis and a generally poorer prognosis.3-5 A similar inverse correlation between expression of the nm23 gene and the metastatic potential of malignant cells has been demonstrated in carcinomas of the stomach,6 liver,7 ovary,8 and prostate.9 However, detailed results have not been documented in those clinical fields.

The purpose of this study was to elucidate the clinical significance of nm23 expression in human gastric carcinomas and to evaluate its influence on the outcome of patients after surgery.

Materials and Methods

Patients

A total of 110 patients with primary gastric cancer who had undergone gastrectomy at Sapporo Medical University between January 1975 and December 1989 were included in this study. All patients were able to be fol-
lowed up in the survival analysis for at least 5 years. The 73 male and 37 female patients ranged in age from 31 to 81 years, with a mean ± SD age of 57.3 ± 10.6. The clinicopathological features were: 41 early cancers and 69 advanced cancers, of which 66 were associated with lymph node metastasis and 44 were not. Of these 110 tumors, 59 were histologically differentiated and 51 were undifferentiated. There were 30 patients with liver metastasis and 18 with peritoneal dissemination included in the analysis.

Specimens
Surgically resected tissues from the 110 primary tumors, 425 lymph nodes with metastasis, and 4 liver metastatic nodules were fixed with 10% buffered formalin (pH 5.9) for 48 h and embedded in paraffin. All sections were cut to 4-μm thickness. Two sections of each primary tumor were made, one passing through the central portion of the tumor and the other through the periphery. The metastatic lymph nodes were sectioned along the sagittal axis and included the hilum. Metastatic loci in the liver were semiserially sectioned. Sections were studied with hematoxylin-eosin (H&E) and anti-nm23 antibody.

nm23 Immunostaining
Sections were deparaffinized in xylene, washed with phosphate-buffered saline (PBS) three times each for 5 min, and immersed in 1% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. The sections were then washed with PBS three times for 5 min and incubated with 30% normal bovine serum albumin (BSA) at room temperature for 60 min to minimize background staining. The samples were incubated overnight at room temperature with anti-nm23 protein polyclonal antibody (1:700) provided by Professor Tawara of the First Department of Pathology, Hiroshima University. The immunoreaction was visualized using a commercially available streptavidin-biotin system (Histofine SAB-PO, Nichirei, Tokyo, Japan). The sections were counterstained with H&E.

Evaluation of nm23 Immunoreactivity
The immunoreactivity of the nm23 antibody against gastric carcinomas was classified into the following four grades: -, no positive cells were found; +, positive cells that stained less intensely than the normal gastric mucosa were seen; ++, positive cells that stained as intensely as the normal mucosa were seen; and +++, positive carcinoma cells that stained more intensely than the normal mucosa were seen. The immunoreactivity of malignant cells found within a single tumor was not always uniform. Therefore, to assess the degree of nm23 expression more objectively within the entire neoplasm, the following evaluation method was developed. First, the percentage of each staining area was measured using a superscripted four-grade classification within an individual section, and the staining grade of the predominant area was judged as synthetic nm23 immunoreactivity (Fig. 1). The superficial mucosal region was excluded at the grading of primary lesions because they sometimes showed nonspecific reactivity. Second, the specimens with nm23 expression grades of − or + were classified into the reduced expression group, and those graded as ++ or ++++, into the nonreduced expression group.

Clinicopathological Relationships
The correlations between nm23 expression grade in the primary lesion and the following clinicopathological features of gastric cancer were examined: tumor location, macroscopic type, tumor size, histological type, quantity of stroma, infiltrating growth pattern (INF pattern), lymphatic invasion, vascular invasion, depth of invasion, lymph node metastasis, liver metastasis, and peritoneal dissemination. The mutual relationship of the following features which significantly correlated with the expression of nm23 was also examined: histological type, vascular invasion, lymph node metastasis, and liver metastasis. The nm23 expression between primary and metastatic lesions was compared, and the cumulative probability of survival following gastrectomy was examined according to the degree of nm23 expression. Finally, a multivariate analysis was performed to assess the prognostic value of the nm23 expression level within the primary tumors. The classification of gastric carcinomas was in accordance with the Japanese Research Society for Gastric Cancer.

Statistics
General group comparisons were made using the \( \chi^2 \) test. The cumulative probability of survival was calculated using the Kaplan-Meier approach and additional comparisons were made using the generalized Wilcoxon test. Factors related to the outcome of patients who remained alive or died as a result of cancer were analyzed by quantification type II multivariate analysis using a commercially available statistics program (Quart 2) on a personal computer (PC-9801, NEC, Tokyo, Japan). A \( P \) value of less than 0.05 was considered statistically significant.