Surg Today
Jpn J Surg (1999) 29:116–120

SURGERY TODAY

Expression of nm23-H1 in Colorectal Cancer: No Association with Metastases, Histological Stage, or Survival

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Abstract: The correlations of nm23-H1 expression in primary cancer lesions with the already confirmed 14 prognostic variables and survival were examined in 52 advanced colorectal cancer patients, because the clinical roles of nm23-H1 expression in the cancer lesions remain controversial. An immunohistochemical expression of nm23-H1 was found in 23 lesions (positive group) but not found in 29 lesions (negative group). No significant difference between the positive and negative groups was found according to 12 clinicopathological variables including vascular invasion, lymph node and liver metastases, and histological stage. The carcinoembryonic antigen levels (21.5 ± 33.4 ng/ml) of the draining venous blood and argyrophilic nucleolar organizer regions score (3.35 ± 1.36 per nucleus) of the cancer cells in the positive group were not significantly diffeent from those (34.1 ± 102.9 ng/ml and 3.32 ± 1.00 per nucleus, respectively) in the negative group. In addition, no significant difference was found in the survival curves or the 5-year survival rates of the positive and negative groups. From these results, it may be concluded that the nm23-H1 expression was not associated with the aforementioned prognostic variables and the prognosis of advanced colorectal cancer patients.

Key Words: nm23-H1 gene expression, colorectal cancer lesion, metastasis, prognostic variable, survival

Introduction

Since Steeg and co-workers[1] first discovered the nm23 gene in 1988, this gene has elicited a great deal of interest regarding the mechanism by which the nm23-H1 protein may suppress the formation of metastasis. Subsequently, a reduced expression of the nm23-H1 gene and/or messenger RNA (mRNA) has been reported to be associated with a poor prognosis in tumor patients and advanced stages or high metastatic potential of tumors.[1–10] On the other hand, other studies have also reported the opposite results in some malignant tumors,[11–16] and even in some identical tumors including breast and gastrointestinal cancers.[13–16] As a result, the clinical role of nm23-H1 expression in tumors remains controversial.

In this study, to investigate the clinical roles of nm23-H1 expression, the correlations of its immunohistochemical expression in primary cancer lesions with the already confirmed 14 prognostic variables, including clinicopathology,[17] proliferative activity of cancer cells,[18–21] and the carcinoembryonic antigen (CEA) levels[22–24] in draining venous blood (dCEA), were examined in advanced colorectal cancer patients.

Materials and Methods

Fifty-two patients with histologically verified advanced colorectal cancer during the 4-year period between 1984 and 1988 at the First Department of Surgery, Kobe University Hospital (Kobe, Japan) were included in this study. In all patients, the draining venous blood was collected to determine the dCEA levels, as already reported by us.[22–24] The resected specimens were fixed in 4% neutral formalin solution and then embedded in paraffin. To avoid a reduction of immunoreactivity, the time of fixation did not exceed 48 h. Histological examinations were routinely carried out using hematoxylin–eosin stain and Elastica van Gieson's stain, and paraffin-embedded blocks containing a central section of cancer lesions were thus selected for the lesions. Twelve clinicopathologic variables including age, tumor diameter, location, gross type, histological type, depth of invasion into the colorectal wall, venous and lym-

Reprint requests to: Y. Tabuchi
(Received for publication on Oct. 20, 1997; accepted on May 15, 1998)

phatic invasion, lymph node and liver metastases, D number (grade of lymph node dissection), and histological stage were examined according to the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus.[17] The dCEA values were determined using the CEA-RIA Diagnostic kit (Abbott, Wiesbahn, Germany).

Immunohistochemically, the expression of nm23-H1 and proliferative activity of cancer cells represented by argyrophilic nucleolar organizer regions (AgNOR) score were examined as follows. Four-μm sections, in parallel with a central section, were made from the paraffin-embedded cancer lesions. The sections were dewaxed in 100% xylene and dehydrated through graded alcohols. The endogenous peroxidase activity was blocked by preincubation with 3% hydrogen peroxide. Any nonspecific binding was also blocked with normal rabbit serum. The sections were then incubated with primary antibody, mouse monoclonal antihuman nm23-H1 antibody (H1-229, 2 μg/ml, Seikagaku, Tokyo, Japan), at 4 °C overnight. Thereafter, the sections were washed with phosphate-buffered saline (PBS) and incubated with biotinylated rabbit antimouse immunoglobulin G (10 μg/ml, Nichirei, Tokyo, Japan) at room temperature for 10 min. After washing with PBS, the sections were incubated with streptoavidin-biotin-peroxidase complex (100 μg/ml, Nichirei, Tokyo, Japan). As a chromogen, 3-3′-diamino-benzene tetrahydrochroride was used with hydrogen peroxide in Tris buffer. The sections were counterstained with hematoxylin. The staining was examined by two of the authors (Y.T. and T.N.) without prior knowledge of the clinical details, and immunoreactivity was graded into two grades: negative reactivity for little or no staining, and positive reactivity for strong staining (Fig. 1), as already reported by us.[25] To evaluate the proliferative activity of the cancer cells, an analysis of AgNOR was performed, as already reported by both Crocker et al.[18] and us.[21] In brief, the staining solution, which consisted of 1 volume of 2% gelatin in 1% formic acid and 2 volumes of a silver nitrate solution, was poured over the deparaffinized sections, and the preparations were then left in the dark for 40 min at 40 °C. The silver solution was washed off using deionized water, and the sections were dehydrated to xylene and mounted. After color photography of the most deeply invasive parts of cancer lesions at the original magnification ×200, the number of AgNOR black dots were counted in 200 cancer nuclei of all the lesions, and the mean number of the dots per nucleus was defined as the AgNOR score for each of the lesions.[21]

The data were analyzed by Student's *t*-test, and either the chi-squared or Fisher's exact probability calculation tests. The survival curves and rates of the positive and negative groups were estimated by the Kaplan-Meier method, and the statistical difference of the survival curves was determined by the log-rank test. *P*-Values of less than 0.05 were considered to be significant.

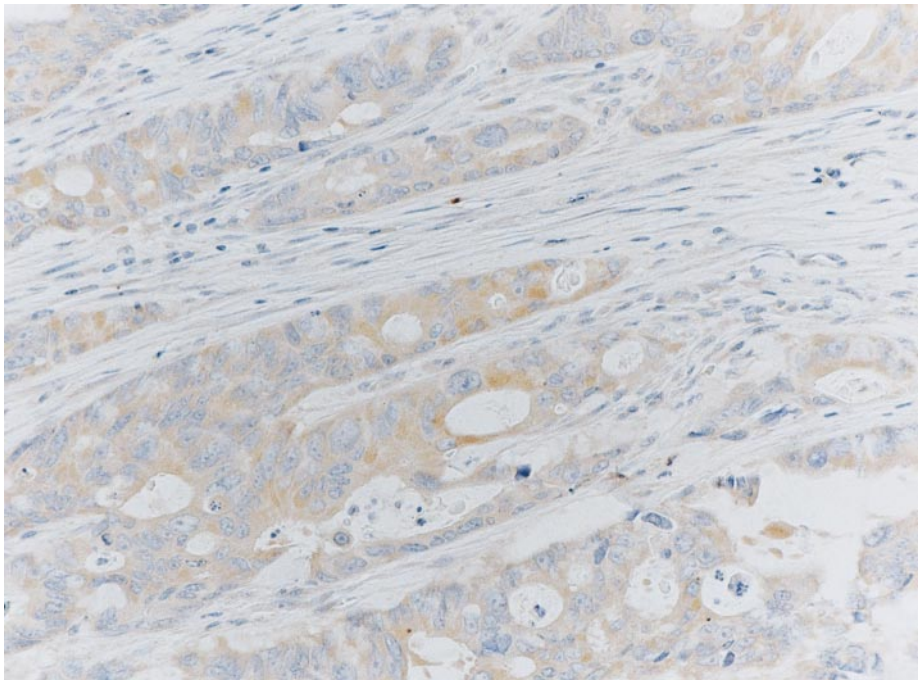

Fig. 1. Immunohistochemical nm23-H1 stain. nm23-H1 expression is found in almost all cancer cytoplasms. Patients with this grade of expression in the cancer lesions were treated as "positive" in this study. Original magnification ×200

Table 1. Correlations of the nm23 expression with clinicopathological variables in colorectal cancer lesions

Clinicopathological variable[a]	Positive group ($n = 23$)	Negative group ($n = 29$)	*P*-Value
Age[b]	64.4 ± 11.9	60.9 ± 10.7	0.276
Diameter (cm)[b]	6.8 ± 2.6	5.8 ± 2.0	0.123
Location			
colon	13	12	
rectum	10	17	0.278
Gross type			
1	2	2	
2	20	26	0.953
3	1	1	
Histological type			
well	15	16	
moderate	6	12	0.428
poor	2	1	
Depth of invasion			
mp-ss (a_1)	10	15	
se(a_2)	9	14	0.655
si (a_i)	4	0	
Venous invasion			
0	10	15	
1	7	6	0.705
2–3	6	8	
Lymphatic invasion			
0	6	13	
1	6	9	0.281
2–3	11	7	
Node metastasis			
0	2	8	
1–2	17	16	0.331
3–4	4	5	
Liver metastasis			
0	20	26	
1–3	3	3	0.547
D number			
0	0	2	
1–2	15	17	0.820
3–4	8	10	
Histological stage			
I–II	1	7	
III	17	18	0.135
IV	5	4	

[a] The prognostic variables are described according to the Japanese Classification of Colorectal Carcinoma (see Ref. 17)
[b] Mean ± standard deviation

Results

Correlations of nm23-H1 Expression with Clinicopathological Variables

nm23-H1 expression was found in 23 cancer lesions (positive group), but not in 29 lesions (negative group). The correlations of the nm23-H1 expression with 12 clinicopathological variables were examined in the positive and negative groups (Table 1). However, no significant difference between the positive and negative groups was found for each of the variables.

Correlations of nm23-H1 Expression with dCEA Levels and AgNOR Score

Regarding the correlations of the nm23-H1 expression with the dCEA levels and AgNOR score, the levels and score in the positive group were not statistically different from those in the negative group (Table 2).

Survival Curves and the 5-Year Survival Rates in the Positive and Negative Groups

As shown in Fig. 2, no significant difference was found in the survival curves of the positive and negative group,

Table 2. Correlations of the nm23-H1 expression with the dCEA levels and the AgNOR score

dCEA levels and AgNOR score[a]	Positive group (n = 23)	Negative group (n = 29)	P-Value
dCEA (ng/ml)	21.5 ± 33.4	34.1 ± 102.9	0.577
AgNOR score	3.35 ± 1.36	3.32 ± 1.00	0.937

dCEA, carcinoembryonic antigen level in draining venous blood; AgNOR, argyrophilic nucleolar organizer regions
[a] Mean ± standard deviation

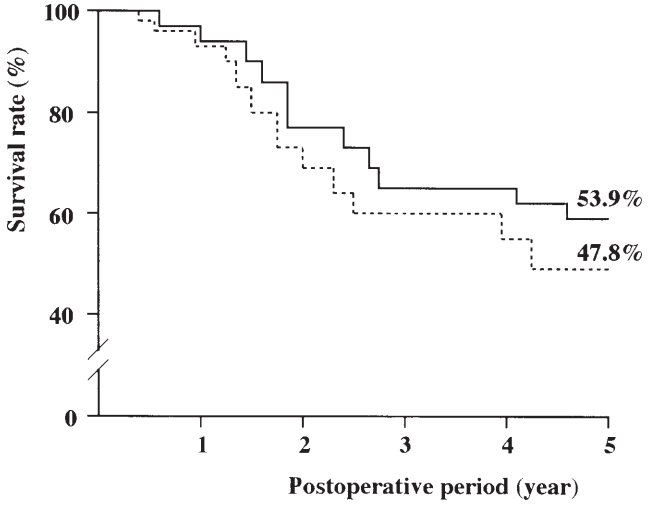

Fig. 2. Survival curves and the 5-year survival rates in the positive and negative groups *Continuous line*, positive group (n = 23); *dashed line*, negative group (n = 29). P = 0.1823 (log-rank test)

although the 5-year survival rate (53.9%) in the positive group was somewhat higher than that (47.8%) in the negative group.

Discussion

The reduced expression of the nm23 gene and/or mRNA has been reported to be associated with a high metastatic potential, advanced stages, and a poor prognosis in various tumors,[2–10] and to be clinically used as one of the prognosticators in various tumors.[2–10] However, the opposite results have also been reported in various tumors[11–16] and even in some identical tumors.[13–16] As a result, the clinical roles of the nm23-H1 expression remain controversial. In this study, the correlations of the immunohistochemical nm23-H1 expression with the already confirmed 14 prognostic variables[17–24] were examined in advanced colorectal cancer patients, to investigate the clinical role of its immunohistochemical expression in the cancer lesions.

The results revealed no association of the immunohistochemical nm23-H1 expression in the cancer lesions with the already confirmed 14 prognostic variables.[17–24] The survival curve in the positive group was not statistically different from that in the negative group, although the 5-year survival rate in the former was somewhat higher than that in the latter. From these results, it may be concluded that the nm23-H1 expression is not associated with prognostic variables including lymph node and liver metastases, vascular invasion and histological stage, dCEA levels, and proliferative activity of cancer cells represented by AgNOR score or with the prognosis of advanced colorectal cancer patients.

Recently, two human nm23 genes have been identified, nm23-H1[26] and nm23-H2,[27] that encode for distinct proteins which are 88% homologous. nm23-H1 and nm23-H2 are identical to human nucleoside-diphosphate kinase (NDPK) A and B, respectively.[28] Although this fact has been attributed to the biologic activity of the nm23 genes, no correlation has been observed between the NDPK enzymatic activity and the suppression of metastasis.[13,29] Based on these facts and/or observations, we therefore conclude that nm23-H1 expression in the advanced colorectal cancer lesions does not appear to be associated with either the already confirmed prognostic variables[17–24] or with the prognosis, as already reported by some investigators.[11–16] However, further precise studies, including prospective control studies on various malignant tumors, may still be necessary to completely elucidate the clinical role of nm23-H1 expression in human tumor lesions.

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