Expression of nm23-H1 Predicts Lymph Node Involvement in Colorectal Carcinoma

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PURPOSE: Reduced expression of the metastasis suppressor gene nm23-H1 has previously been correlated with high tumor metastatic potential and fatal clinical outcome in some tumors (e.g., breast). For colorectal carcinomas, the findings are equivocal. METHODS: We have used a monoclonal antibody against nm23-H1 to investigate the expression in colorectal carcinomas at the time of primary curative surgery (R0 resection) to assess if there was any relation between nm23-H1 expression and stage or histologic grade at the time of primary tumor removal. RESULTS: Of 100 colorectal carcinomas studied (Stages I, II, and III according UICC, all resected curatively), nm23-H1 immunoreactivity was weak in 41 (41 percent), moderate in 24 (24 percent), and strong in 35 (35 percent) cases. The grade of positivity against nm23-H1 was significantly lower in advanced stages of the disease (Stages II or III) (P < 0.001, chi-squared = 52.8). In tumors with low or weak immunoreactivity against nm23-H1, frequency of lymph node metastases was significantly higher compared with those with moderate or strong staining (P < 0.001; chi-squared = 50.58). Therefore, with a sensitivity of 93 percent and a specificity of 58 percent, low nm23-H1 immunoreactivity of the primary tumor, assessed at the time of surgery, is an indicator of the presence of lymph node metastases. CONCLUSIONS: Immunohistochemical evaluation of nm23-H1 in the primary tumor or in a biopsy is a useful predictor of stage of disease and presence of lymph node metastases in colorectal carcinomas and may have clinical significance, e.g., in predicting optimal therapeutic regimes. [Key words: nm23-H1; Colorectal carcinoma; Staging; Lymph node metastases]


Identification of multiple clinical and pathologic prognostic factors in colorectal carcinomas has permitted a reasonable degree of risk stratification. However, all clinical and pathologic data fail to predict the actual virulence of a tumor concerning tumor relapse or metastases to lymph nodes or distant organs in individual cases. The nm23-H1 gene product has been identified as a potential metastases suppressor, which has a structural homology to a nucleoside diphosphate kinase (for review, refer to Steeg et al.1). Studies in several types of rodent tumors and human breast carcinomas have shown an inverse correlation between nm23-H1 expression and prognosis.2,3 For colorectal carcinomas, onset of metastasis is related to loss or mutation of the nm23-H1 gene,4 and somatic allelic deletion has been reported to be homozygous in lymph node metastases.5-7 However, the relationship between nm23-H1 expression and prognosis or histopathologic parameters has not yet been examined in a large series of tumors. To date, all reported studies are based on small numbers of patients and have used different techniques for nm23-H1 assessment, and little attempt has been made to assess its predictive value. The latter is an important concept because it is becoming clear that identification of specific molecular defects may be used to predict not only prognosis but also optimal treatment regimes.

We, therefore, assessed nm23-H1 immunoreactivity in a series of surgically removed primary colorectal carcinomas to establish whether there were correlations between degree of expression in the primary tumor and histopathologic data including tumor grade, stage, and lymph node involvement.

METHODS

For analysis of nm23-H1, tissue blocks of paraffin-embedded material were obtained from 100 consecutive, nonselected patients with primary colorectal carcinoma, operated on in the Department of Surgery of the University of Erlangen-Nürnberg, Germany between 1991 and 1993. No patient had received preoperative chemotherapy or radiotherapy. All patients were operated curatively (R0-resections), defined as no residual tumor detectable by presently available and generally accepted diagnostic methods including histologic examinations of resection margins.8 Radical
surgical tumor resection with regional en bloc lymphadenectomy proximally up to the origin of the vascular trunks (i.e., ileocolic, right, middle, and left colic or inferior mesenteric vessels) was performed in every patient. Tumor typing, grading, and staging was performed using World Health Organization and UICC criteria. To ensure accurate and complete staging, an average of 21 lymph nodes were examined for each tumor, with a minimum of 12 nodes in each case. Twelve carcinomas (12 percent) were in Stage I, 43 (43 percent) in Stage II, and 45 (45 percent) in Stage III (according to UICC). Ten (10 percent) were assessed as well differentiated, 62 (62 percent) as moderately differentiated, and 28 (28 percent) as poorly differentiated colorectal carcinomas.

For immunohistochemistry, 4-μm sections of routinely fixed paraffin-embedded tissue of the primary colorectal carcinoma was deparaffinized in xylene and then rehydrated. Endogenous peroxidase activity was blocked with 1 percent hydrogen peroxide in methanol for 30 minutes. After a short rinse of phosphate-buffered saline (PBS), sections were preincubated with normal goat serum to reduce nonspecific staining. After removing the blocking solution, slides were incubated with a 1:200 dilution of primary antibody (nm23 Ab-1, Clone NM301, obtained from Oncogene Science, Cambridge, MA) at room temperature overnight. Sections were washed with PBS and incubated with biotinylated goat antibody to mouse immunoglobulin (Dakopatts, Glostrup, Denmark) and then covered with streptavidin-biotin-peroxidase complex (Dakopatts) for 30 min each step, with washing in PBS between steps. The peroxidase reaction was allowed to proceed for 8 min, with 0.05 percent 3,3-diaminobenzidine tetrahydrochloride solution as substrate in PBS with 0.02 percent hydrogen peroxide. Sections were counterstained with hematoxylin and mounted. Sections known to stain positively were included in each run, and negative controls were also performed by replacing the primary antibody with mouse ascites fluid (Sigma-Aldrich Biochemicals, St. Louis, MO).

Two sections from two different paraffin-embedded tissue blocks were stained for each tumor. Slides were examined and scored independently by two of the authors (CW, AT) without any clinical or pathologic information. Degree of nm23-H1 positivity was classified into three categories by semiquantitative estimation of the proportion of positive staining tumor cells on the entire slide (absent or weak, less than 30 percent of positive tumor cells; moderate, 30–60 percent of positive tumor cells; strong, more than 60 percent of positive tumor cells).

We tested the significance of difference in frequency of nm23-H1 immunoreactivity and histopathologic data using the chi-squared test. Contingency tables and chi-squared test (according to Pearson, with ordered categories) were used to evaluate relations between nm23-H1 positivity and T and N categories, grade of tumor differentiation, and stage of disease.

RESULTS

Among the 100 colorectal carcinomas examined, 41 (41 percent) showed weak, 24 (24 percent) moderate, and 35 (35 percent) strong immunoreactivity for nm23-H1. nm23-H1 was observed in the cytoplasm and was predominantly perinuclear. The staining pattern was nearly homogeneous and comparable in almost all parts of the tumor. For illustration of staining pattern, please see Figure 1.

When comparing nm23-H1 expression with stage of disease at time of surgery, a highly significant correlation could be established: 85 percent (35/41) of the tumors, which showed no or only weak immunoreactivity, belonged to Stage III, whereas only 9 percent (3/35) of tumors that exhibited strong staining wereStage III (P < 0.001).

Because pathologic staging criteria are based on pTNM (pathologic tumor, nodes, metastasis) classification, we further examined the relationship between nm23-H1 expression and pT and N categories. Because of the small number of patients with pT1 tumors (infiltration of submucosa only), it was not possible to show a significant correlation between nm23-H1 staining and pT category.

Nevertheless, an inverse trend between pT and nm23-H1 expression was observed. Staining was less intense in tumor parts that exhibited higher depth of infiltration.

In contrast to the pT category of tumors, lymph node status was significantly related to nm23-H1 expression (P < 0.001). From 45 patients who had lymph node metastases at the time of surgery, 42 (93 percent) had weak or moderate immunostaining for nm23-H1, whereas only three patients (7 percent) exhibited strong staining (chi-squared value, 50.6; P < 0.001) (Table 1). For practicability and simplification of interpretation of immunohistochemical results, staining intensities were categorized into two groups, weak and moderate vs. strong immunoreac-