Association of Low $nm23$ RNA Levels in Human Primary Infiltrating Ductal Breast Carcinomas with Lymph Node Involvement and Other Histopathological Indicators of High Metastatic Potential

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ABSTRACT

Expression of a recently identified murine gene, $nm23$, has been previously proposed to be inversely correlated to tumor metastatic potential in rodent model systems. The present study was designed to investigate whether $nm23$ RNA was detectable in human tumor tissue, and if it was differentially expressed. $nm23$ RNA levels in 27 human primary infiltrating ductal breast carcinomas were determined by using Northern blots or in situ hybridization. These data were compared to traditional histopathological indicators of metastatic potential, including the number of involved (tumor bearing) lymph nodes, grade of differentiation, and hormone receptor status. A striking consistency was observed in all tumors from patients with involved lymph nodes. Using Northern blot or in situ hybridizations, all of these tumors expressed low levels of $nm23$ RNA. Quantitative in situ hybridization on tumors from patients with 0 involved lymph nodes identified two groups: (a) approximately 75% contained high $nm23$ RNA levels, and (b) 25% contained significantly $(a = 0.05)$ lower $nm23$ RNA levels. Low $nm23$ RNA levels in the 0 involved lymph node tumors were accompanied by two additional histopathological indicators of high metastatic potential, low nuclear and cytoplasmic estrogen receptor content, and poorly differentiated histological grade. In contrast, none of the high $nm23$ RNA level tumors were receptor negative and poorly differentiated. We conclude that $nm23$ RNA levels are differentially expressed in human breast tumors, and that low $nm23$ RNA levels are associated with histopathological indication of high metastatic potential. Short term (median follow-up of 16 months) clinical course data were consistent with $nm23$ RNA levels, in that 2 of 11 low $nm23$ RNA content patients (including one from the 0 involved lymph node group) developed metastases, while none of the high $nm23$ RNA patients have experienced recurrent disease.

INTRODUCTION

The accurate prediction of tumor metastatic potential, the probability that cells from a primary tumor will move to and colonize distant organs, is an important goal in clinical oncology. For patients with solid, malignant tumors, metastases are the leading cause of death. Accurate predictive information can be critical to therapeutic choices and provides the patient with realistic prognostic information. We have recently identified a novel murine gene, $nm23$, that is associated with tumor metastatic potential $(1)$. In four rodent experimental systems, tumor cells of low metastatic potential contained significantly greater $nm23$ RNA levels than related, highly metastatic tumor cells: (a) in a series of cell lines, all derived from a single K-1735 melanoma, two low metastatic cell lines contained 10-fold greater $nm23$ RNA levels than did five related, high metastatic cell lines $(1)$; (b) in rat nitrosomethylurea-induced mammary tumors, nonmetastatic tumors contained higher $nm23$ RNA levels than did primary metastatic mammary tumors or pulmonary metastases $(1)$; (c) nonmetastatic rat embryo fibroblasts cotransfected with $c-Ha-ras$ and adenovirus $Ela$ contained significantly greater $nm23$ RNA content than did highly metastatic ras-transfected rat embryo fibroblast lines $(2)$; (d) low metastatic potential primary tumors arising in BALB/c mice carrying the RIII strain of MMTV$^3$ contained 3-fold greater $nm23$ RNA levels than did highly metastatic primary tumor from BALB/c mice carrying the C3H strain of MMTV $(3)$. The expression and function of the $nm23$ gene in human cancer has not yet been evaluated. If inversely correlated to metastatic potential in some human cancers, this gene could serve as a supplement to histopathological diagnostic techniques currently in use.

As a first step toward this research goal, we have asked whether (a) $nm23$ RNA was detectable in human cells; (b) $nm23$ RNA levels were differentially expressed in human tumors; and (c) $nm23$ RNA levels were correlated to accepted histopathological indicators of tumor metastatic potential. Breast cancer was selected for study for two reasons: first, two animal experimental metastases systems (nitrosomethylurea- and MMTV-induced tumors) in which $nm23$ RNA levels were differentially expressed were mammary tumors. Second, a battery of histopathological criteria are available for predicting the metastatic potential of primary infiltrating ductal carcinomas and can serve as a reference point for comparison to $nm23$. The most widely accepted criterion is the patient's number of involved (tumor bearing) axillary lymph nodes at surgery. Statistically, 25% of women with 0 involved lymph nodes at surgery develop metastases within 10 years, while 65 and 86% of patients with 1–3 involved nodes and 4+ involved nodes, respectively, develop metastases in the same period $(4)$. Lymph node involvement data can be supplemented by quantitation of tumor hormone receptor levels, cytology, and nuclear grade $(5)$.

Two possible sources of tumors were considered in the design of this study. Tumor banks contain large numbers of tumors and have been utilized for studies at the DNA level $(6)$. Some of these banks have relatively long follow-up periods, and permit comparison to both histopathological criteria and patient clinical course. However, RNA is much more susceptible to degradation than DNA. To ensure accurate quantitation of $nm23$ RNA levels, we determined that the samples must be handled and stored under RNase-free conditions (i.e., rapid freezing in liquid nitrogen, storage in RNase-free containers), which are unavailable in virtually all tumor banks. Therefore, this study was designed to utilize tumors recently collected at the University of Pisa, Italy, under strict RNase-free conditions. We report that $nm23$ RNA levels are differentially expressed in human primary infiltrating ductal carcinomas, and that tumor cell $nm23$ RNA levels were correlated with lymph node metastases and other histopathological indicators of metastatic potential.

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1 G. P. was partially funded by the Italian Association for Cancer Research (AIRC).

2 To whom requests for reprints should be addressed, at Building 10, Room 2A33, NIH, Bethesda, MD 20892.

3 The abbreviation used is: MMTV, mouse mammary tumor virus.
of each tumor were hybridized in situ; one set of sections was incubated in radiographic emulsion for 7 days, and the other set for 14 days. At least two separate in situ hybridization experiments were performed on each tumor from 7-day exposures of each experiment. Each photomicrograph contained approximately 50 cells, a percentage of which were difficult to analyze due to overlapping. The number of grains in the cytoplasm associated with the nuclear area for 20 tumor cells in each photomicrograph was determined. Mean, standard error, and 95% confidence intervals were calculated from these data. We have observed that, for each tumor, mean and standard deviation data from different sections of a tumor did not vary significantly (data not shown). Additionally, the percentage of cells with zero grains of nm23 RNA was tabulated. Similar means of data analysis have been reported elsewhere for in situ hybridizations with different probes (10-12).

For primary infiltrating ductal carcinomas were fixed, paraffin embedded, and sectioned by standard techniques. Sections were applied to RNase-free slides previously coated with 5 μg/ml poly-L-lysine. Sections were then deparaffinized in xylene and hydrated. The slides were incubated serially in 5 mM MgCl₂ in phosphate-buffered saline for 5 min; 5 μg/ml proteinase K in 10 mM Tris, pH 7.5, for 10 min at 37°C; 400:1 (v/v) triethanolamine:acetic anhydride for 10 min; 0.1 M glycine in 0.2 M Tris, pH 7.4, for 10 min; and phosphate-buffered saline overnight. Sections were incubated in a Northern blot prehybridization buffer (2) for 3 h at 37°C. The pNM23 riboprobe insert was [³²P]-labeled, and hybridization and initial washing of the slides were performed as described (13). Remaining unhybridized riboprobe was then digested by incubation in 50 μg/ml ribonuclease A, 250 units/ml RNase T1 in 2× standard saline citrate at 37°C for 45 min, followed by a wash in 2× standard saline citrate. Slides were dehydrated and processed for autoradiography as described (13).

RESULTS

Northern Blot Analyses. Total cellular RNA was extracted from 10 primary infiltrating ductal breast carcinomas and 4 fibroadenomas and probed for nm23 RNA content by using Northern blot hybridization (Fig. 1). With regard to the infiltrating ductal carcinomas, nm23 RNA levels were plotted against the patient's number of involved (tumor bearing) lymph nodes at surgery. Lymph node involvement data are considered to be the single most accurate predictor of metastatic potential. Statistically, the percentage of patients who develop metastases within 10 years of surgery is 24% for 0 involved lymph nodes, 64.5% for 1-3 involved lymph nodes, and 86.2% for 4 or more involved lymph nodes (4). All tumors from 0 involved lymph node patients in our study contained hybridizable nm23 RNA levels; the size of the band on Northern blots (0.8 kilobase) compares closely with data in murine and rat systems (1, 2). Significantly, lower levels of nm23 RNA were present in all tumors from involved lymph node patients, expected to be high metastatic potential. With regard to other histopathological indicators of metastatic potential, no significant correlations were observed between nm23 RNA levels and primary tumor...
nm23 RNA LEVELS IN HUMAN BREAST CANCER

Infiltrating Ductal Carcinoma

No Involved Lymph Nodes Involved Lymph Nodes

0/26 5/33

Involved/Total Lymph Nodes Examined

Fig. 1. Quantitation of human fibroadenoma and primary breast carcinoma nm23 RNA levels by using Northern blot hybridization. Total cellular RNA was extracted from 14 specimens in a total of three experiments (!, O, C), RNA levels determined by Northern blot hybridization, and the blots were exposed to X-ray film for 2 days. A, correlation of nm23 RNA levels and metastatic potential. Hybridizable RNA levels to a pNM23-1 probe were determined by densitometry and were graphed by tumor type and lymph node status. B, representative Northern blot of the pNM23-1 complementary DNA insert (top) and a pLR21 laminin receptor complementary DNA insert (bottom) to one set of RNAs graphed in A (C).

The variability of nm23 RNA levels observed in the infiltrating ductal carcinomas from 0 involved lymph node patients prompts an additional question. Since 24% of the patients in this category develop metastatic disease statistically, do the observed differences in nm23 RNA levels identify the subpopulation of these patients with high metastatic potential tumors? One hypothesis is that those tumors with lower nm23 RNA levels in Fig. 1 represent the high metastatic potential subpopulation. Alternatively, breast tumors contain, to varying degrees, normal stromal cells, lymphocytes, and vascular endothelial cells. We have found that normal tissues express significant amounts of nm23 RNA (data not shown), and the differences observed in hybridizable nm23 RNA levels could therefore reflect the variable presence of normal cells. To eliminate the latter possibility, the nm23 RNA levels of tumor cells within infiltrating ductal carcinomas were determined by in situ hybridization, and correlated to histopathological indicators of metastatic potential.

In Situ Hybridizations. In the experiments shown in Figs. 2–4 and Table 1, paraffin-embedded sections of 17 additional infiltrating ductal carcinomas were hybridized in situ to a"
nm23 RNA LEVELS IN HUMAN BREAST CANCER

**Table 1 Summary of histopathological, clinical course, and nm23 data**

<table>
<thead>
<tr>
<th>Biopsy number</th>
<th>No. of involved lymph nodes/total examined</th>
<th>Tumor diameter (cm)</th>
<th>Age at surgery (yr)</th>
<th>Nuclear</th>
<th>Cytoplasmic</th>
<th>Histological grade*</th>
<th>Disease progression</th>
<th>Mean grains</th>
<th>95% confidence limits</th>
<th>% of tumor cells with 0 grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>4627/86(C)</td>
<td>0/22</td>
<td>3.0</td>
<td>66</td>
<td>80% 3+</td>
<td>70% 3+</td>
<td>MWD*</td>
<td>None</td>
<td>5.2</td>
<td>3.2-7.2</td>
<td>0</td>
</tr>
<tr>
<td>5904/86(C)</td>
<td>0/22</td>
<td>3.5</td>
<td>67</td>
<td>70% 3+</td>
<td>80% 3+</td>
<td>PD</td>
<td>None</td>
<td>3.2</td>
<td>2.6-3.8</td>
<td>0</td>
</tr>
<tr>
<td>19,183/86(C)</td>
<td>0/46</td>
<td>7.0</td>
<td>64</td>
<td>70% 2+</td>
<td>60% 2+</td>
<td>PD</td>
<td>None</td>
<td>2.8</td>
<td>2.3-3.2</td>
<td>0</td>
</tr>
<tr>
<td>4691/87(A)*</td>
<td>0/11</td>
<td>2.0</td>
<td>60</td>
<td>Negative</td>
<td>7% 1+</td>
<td>PD</td>
<td>Cutaneous recurrence</td>
<td>0.7*</td>
<td>0.4-1.0</td>
<td>45</td>
</tr>
<tr>
<td>5087/87(A)</td>
<td>0/5</td>
<td>2.0</td>
<td>52</td>
<td>Negative</td>
<td>Negative</td>
<td>MWD</td>
<td>None</td>
<td>3.4</td>
<td>2.7-4.1</td>
<td>0</td>
</tr>
<tr>
<td>5355/87(A)</td>
<td>0/17</td>
<td>2.0</td>
<td>52</td>
<td>10% 1+</td>
<td>90% 3+</td>
<td>MWD</td>
<td>None</td>
<td>6.5</td>
<td>5.2-7.8</td>
<td>0</td>
</tr>
<tr>
<td>14,839/86(B)*</td>
<td>0/30</td>
<td>3.0</td>
<td>66</td>
<td>Negative</td>
<td>8% 1+</td>
<td>PD</td>
<td>None</td>
<td>1.1*</td>
<td>0.6-1.7</td>
<td>28</td>
</tr>
<tr>
<td>14,762/86(B)</td>
<td>0/27</td>
<td>2.5</td>
<td>48</td>
<td>Negative</td>
<td>10% 1+</td>
<td>MWD</td>
<td>None</td>
<td>7.1</td>
<td>6.2-8.0</td>
<td>0</td>
</tr>
<tr>
<td>5763/87(A)</td>
<td>1/16</td>
<td>4.0</td>
<td>49</td>
<td>Negative</td>
<td>5% 1+</td>
<td>PD</td>
<td>None</td>
<td>1.2</td>
<td>0.7-1.7</td>
<td>33</td>
</tr>
<tr>
<td>12,990/86(B)</td>
<td>4/25</td>
<td>2.5</td>
<td>67</td>
<td>Negative</td>
<td>10% 1+</td>
<td>MWD</td>
<td>None</td>
<td>0.3</td>
<td>0.3-0.5</td>
<td>76</td>
</tr>
<tr>
<td>18,637/86(B)</td>
<td>6/44</td>
<td>2.3</td>
<td>65</td>
<td>40% 1+</td>
<td>60% 1+</td>
<td>PD</td>
<td>None</td>
<td>0.8</td>
<td>0.3-1.3</td>
<td>55</td>
</tr>
<tr>
<td>13,599/86(C)</td>
<td>7/20</td>
<td>2.5</td>
<td>39</td>
<td>30% 1+</td>
<td>50% 2+</td>
<td>WD</td>
<td>None</td>
<td>0.9</td>
<td>0.6-1.4</td>
<td>30</td>
</tr>
<tr>
<td>13,933/86(C)</td>
<td>10/39</td>
<td>4.7</td>
<td>47</td>
<td>Negative</td>
<td>80% 1+</td>
<td>PD</td>
<td>None</td>
<td>0.5</td>
<td>0.2-0.8</td>
<td>55</td>
</tr>
<tr>
<td>5651/87(A)</td>
<td>10/16</td>
<td>4.0</td>
<td>64</td>
<td>Negative</td>
<td>20% 2+</td>
<td>WD</td>
<td>None</td>
<td>0.7</td>
<td>0.3-1.1</td>
<td>50</td>
</tr>
<tr>
<td>4165/87(A)</td>
<td>11/14</td>
<td>1.5</td>
<td>62</td>
<td>70% 3+</td>
<td>50% 3+</td>
<td>PD</td>
<td>None</td>
<td>0.6</td>
<td>0.2-1.0</td>
<td>55</td>
</tr>
<tr>
<td>5725/87(A)</td>
<td>15/15</td>
<td>4.0</td>
<td>77</td>
<td>40% 1+</td>
<td>80% 3+</td>
<td>MWD</td>
<td>None</td>
<td>0.7</td>
<td>0.4-1.1</td>
<td>48</td>
</tr>
<tr>
<td>12,573/86(C)</td>
<td>34/38</td>
<td>1.0</td>
<td>69</td>
<td>50% 1+</td>
<td>40% 1+</td>
<td>PD</td>
<td>Bone metastasis</td>
<td>0.7</td>
<td>0.3-1.1</td>
<td>55</td>
</tr>
</tbody>
</table>

* From the University of Pisa, Italy. Tumors were grouped into 3 sets (A, B, C) due to technical limitations on the number of slides per experiment (see "Material and Methods").

* Determined by immunocytochemical methods as described (12-15).

* Determined by microscopic analysis of hematoxylin-eosin-stained sections, blind to other patient data.

* MWD, moderately well differentiated; PD, poorly differentiated; WD, well differentiated. Based on median follow-up period of 16 months.

* Identifies tumors with low nm23 RNA levels from 0 involved lymph node patients.

* Patient died of cause unrelated to cancer.

RNA in each tumor versus the patient's number of involved lymph nodes at surgery; Table 1 lists the 95% confidence intervals for these data. All tumors from patients with involved lymph nodes contained tumor cells with extremely low nm23 RNA levels. In 6 of 8 (75%) of the tumors from 0 involved lymph node patients, significantly greater (a = 0.05) tumor cell nm23 RNA levels were observed, consistent with low metastatic potential. However, 2 of 8 (25%) of the 0 involved lymph node tumors (Table 1; biopsies 4691 and 14839) contained tumor cells with significantly less nm23 RNA than the other tumors in this group (a = 0.05). nm23 RNA levels were not significantly different from those of the involved lymph node group (a = 0.05). Fig. 4 shows photomicrographs from a representative experiment that includes two tumors from 0 involved lymph node patients, one with high and one with low levels of nm23 RNA. As shown, the nm23 RNA expression of biopsy 14839 tumor closely matches that of the involved lymph node tumors.

Given the fact that 24% of women with 0 involved lymph nodes develop metastatic cancer (4), we investigated additional histopathological predictors of metastatic potential and patient clinical course for the tumors studied by the in situ hybridization technique (Table 1). These data address the question of whether the 25% of the 0 involved lymph node tumors with low nm23 RNA levels were of high metastatic potential. As shown in Table 1, each of the tumors in question was negative for nuclear estrogen receptor, less than 10% were positive for cytoplasmic estrogen receptor, and of poorly differentiated histological grade, all parameters indicative of high tumor metastatic potential (5). Additionally, over a median of 16 months, one of the two patients in this subgroup had developed a cutaneous metastasis. Of the 6 patients with 0 involved lymph nodes and significantly higher nm23 RNA levels, none had both low estrogen receptor and poorly differentiated histological grade, or developed metastases over the same follow-up period. The data support the conclusion that low tumor cell nm23 RNA levels correlate with primary breast tumors with histopathological evidence of high metastatic potential.

The in situ hybridization data were also analyzed for heterogeneity. As shown, the nm23 RNA expression of biopsy 14839 tumor closely matches that of the involved lymph node tumors.

pNM23 riboprobe. Tumor cell nm23 RNA levels were quantitated and compared with histopathological indicators of metastatic potential. Fig. 2 shows the hybridization of the pNM23 riboprobe sense and "antisense" RNA transcripts to sections of one tumor, demonstrating the specificity of the in situ hybridization technique. The antisense transcript, complementary to cellular nm23 RNA, exhibited significant hybridization as evidenced by grains deposited during autoradiography, while the sense transcript of the same plasmid, which is identical to nm23 RNA, did not hybridize. Fig. 3 graphs the mean ± SEM grains of tumor cell nm23 RNA in each tumor versus the patient’s number of involved lymph nodes at surgery; Table 1 lists the 95% confidence intervals for these data. All tumors from patients with involved lymph nodes contained tumor cells with extremely low nm23 RNA levels. In 6 of 8 (75%) of the tumors from 0 involved lymph node patients, significantly greater (a = 0.05) tumor cell nm23 RNA levels were observed, consistent with low metastatic potential. However, 2 of 8 (25%) of the 0 involved lymph node tumors (Table 1; biopsies 4691 and 14839) contained tumor cells with significantly less nm23 RNA than the other tumors in this group (a = 0.05). nm23 RNA levels were not significantly different from those of the involved lymph node group (a = 0.05). Fig. 4 shows photomicrographs from a representative experiment that includes two tumors from 0 involved lymph node patients, one with high and one with low levels of nm23 RNA. As shown, the nm23 RNA expression of biopsy 14839 tumor closely matches that of the involved lymph node tumors.

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The in situ hybridization data were also analyzed for hetero-
Fig. 4. Representative in situ hybridization experiment. Photomicrographs of an in situ hybridization to one of three sets of breast tumors (Table 1, group B) graphed in Fig. 2 are shown. Indicated below each photomicrograph are the biopsy number and the number of involved (tumor-bearing)/total lymph nodes examined during surgery.

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Involved/Total Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14762/86</td>
<td>0/27</td>
</tr>
<tr>
<td>14839/86</td>
<td>0/30</td>
</tr>
<tr>
<td>12390/86</td>
<td>4/25</td>
</tr>
<tr>
<td>18637/86</td>
<td>6/44</td>
</tr>
</tbody>
</table>

Gene expression in nm23 RNA levels in human breast cancer. Because of the observations that only a small percentage of primary tumor cells form metastases and that cells within a primary tumor are heterogeneous for many characteristics (18, 19), it was possible that tumor metastatic potential actually depended on the presence or absence of a subpopulation of high metastatic potential tumor cells, regardless of mean data. The percentage of tumor cells with zero grains nm23 RNA, the expected subpopulation with high metastatic potential, was tabulated for each tumor and is listed in Table 1. As shown, this method of analysis identified the same tumors as having high metastatic potential as did mean nm23 RNA expression data. Therefore, by both mean and subpopulation analyses, breast carcinomas containing tumor cells with low nm23 RNA content were of high metastatic potential.

DISCUSSION

Three major conclusions can be drawn from the data presented herein: (a) nm23 RNA can be detected in human tissue by using a murine complementary DNA probe under relatively stringent hybridization and washing conditions, indicating the existence of a conserved human nm23 gene. (b) nm23 RNA levels were differentially expressed between different primary infiltrating ductal breast carcinomas, when assayed by Northern blot or in situ hybridization. Differences in nm23 RNA levels were quantitative, not qualitative, in agreement with data in four rodent metastasis systems (1–3). (c) Low tumor cell nm23 RNA levels correlated with histopathological indicators of high metastatic potential, including lymph node involvement, hormone receptor status, and differentiation. Involved lymph nodes are the primary histopathological prognostic indicator used for infiltrating ductal carcinomas, and all tumors from involved lymph node patients (expected to be of high metastatic potential) were low in nm23 RNA content. Within the 0 involved lymph node group, nm23 RNA levels compared closely to prognostic information. Statistically 24% of women in this category will develop metastases over 10 years; 25% of the patients in this category had low nm23 RNA levels. Further, those low nm23 RNA content tumors (from 0 involved lymph node patients) exhibited two other histopathological indicators of high metastatic potential: low estrogen receptor content and poorly differentiated cytology. In contrast, none of the tumors with high nm23 RNA levels exhibited both of these latter histopathological criteria. Limited clinical course data are consistent with our conclusions, in that 18% (2 of 11) of the low nm23 RNA level patients (including one from the 0 involved lymph node category) developed metastases over a median 16-month follow-up period. None of the patients with high nm23 RNA levels had developed metastases. Thus, the nm23 gene is differentially expressed with predicted tumor metastatic potential in a human cancer.

The existence of metastases-suppressive genes was postulated from studies of the fusion products of metastatic and nonmetastatic tumor cells (20). Further studies in which activated ras, which is capable of inducing metastatic potential upon transfection (21, 22), failed to induce metastatic potential in certain cells and tumors (22), supported this hypothesis. Pozzatti et al.
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(23) identified the exogenously added adenovirus 2 Eta onco-
gene as the first suppressor gene for ras-induced metastasis. We have reported that nm23 RNA levels are increased in conditions where metastasis suppression has been postulated, i.e., ras and Eta cotransfected rat embryo fibroblasts (2). These data identify the nm23 gene as the first nonimmunologically related cell-derived gene associated with metastasis suppression in rodent systems. The down regulation of the nm23 gene in breast tumors of predicted high metastatic potential, described in this report, indicates that the nm23 gene is also regulated in a human cancer. These data compare to data in retinoblastoma, where mRNA levels for the tumorigenesis suppressor RB gene were demonstrated to be decreased in retinoblastoma, as op-
posed to normal retinal tissue (24). We note that additional data, including chromosomal abnormalities and transfection (25), also support the RB hypothesis. Whether or not the nm23 gene has any metastasis suppressor activity will be determined by transfection experiments.

For infiltrating ductal breast carcinoma, current doctrine advocates chemotherapy for all patients, because of the inability of current prognostic methods to accurately identify primary tumors from lymph node-negative women with high metastatic potential. However, because of the side effects of chemotherapy, an important goal in clinical oncology remains the development of better predictive techniques, in order to spare 76% of women without involved lymph nodes chemotherapy. Recent studies suggest that amplification of the chromosomal erb-b-2 and/or int ongenes may have prognostic value (6, 26). The present study raises the possibility that nm23 RNA levels may also be of prognostic usefulness and indicates the need for large-scale prospective and retrospective studies. Such studies will require material preserved in a RNase-free manner and the use of in situ hybridization to eliminate possible artifacts of normal cells. Further modifications to the in situ hybridization protocol would be required to permit better standardization of assays and quantitation. In situ hybridizations using biotinylated probes have been reported for several genes (13). This technique, which could simplify and accelerate the in situ hybridization protocol, has been unsatisfactory in our laboratory, presumably due to the relatively low abundance of nm23 mRNA. Our preliminary data also suggest that nm23 RNA levels are differentially expressed in colon carcinomas (data not shown), raising the possibility that nm23 gene expression may be of prognostic usefulness in other cancers as well. Our current efforts are directed toward the development of antisera to the nm23 gene product, to determine if nm23 protein levels may also be of prognostic value.

REFERENCES

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