Expression of nm23 in the Primary Tumor and the Metastatic Regional Lymph Nodes of Patients with Gastric Cardiac Cancer

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ABSTRACT
Tumor recurrence and distant metastasis are major causes of treatment failure in gastric cardiac cancer (GCC). Rapid growth of tumor cells and reduced expression of nm23, a metastatic suppressor gene, in tumor cells have been suggested as two important mechanisms for disease progression of GCC. Therefore, to determine the prognostic value of nm23 expression in GCC, we used immunohistochemistry to examine the expression of nm23 in the pathological sections of both gastric cancer and metastatic lymph nodes from 24 stage III patients. Twenty-two patients had total gastrectomy, and two patients had proximal subtotal gastrectomy with a D2 dissection. Postoperative adjuvant therapy was provided, and the clinical responses were followed routinely. Clinical correlation was evaluated by \( \chi^2 \) with Fisher’s exact test and survival by log-rank test. Our results show that the reduced nm23 expression in the primary tumor and in the nodal metastasis is the most useful marker for the poor prognosis of GCC following surgery.

INTRODUCTION
GC is the fourth leading cause of cancer death in Taiwan (1). The annual incidence rate has varied from 3 per 100,000 men to 25 per 100,000 men, depending upon genetic vulnerability, diet, and environmental factors. Although surgery is a curative treatment for the early-stage GC, most patients present with advanced disease (2–6). Furthermore, poor prognosis is compounded by the rapid growth of tumor and spreading of cancer cells as well as disease-associated malnutrition and cachexia. It is worth noting that the prognosis of patients with GCC is worse than that of patients with distally located GC (7). The effort of multiple therapeutic modalities was not beneficial for patients at the late stage (8–12). Therefore, a method for identifying the disease progression of GCC and the potential of cancer cell spreading is important for commencing early treatment and increasing survival.

Rapid growth of tumor cells has been suggested as the major cause for treatment failure in GC surgery (13, 14). It has also been associated with tumor vascularity as well as proliferation-dependent tumor recurrence and distant metastasis (8–12). Nonetheless, patients at the early stage could have rapid tumor recurrence following surgery, and patients at the late stage could survive for a long period of time after treatment, suggesting that intrinsic factor(s) could play a role in this difference.

The increasing evidence has indicated that nm23 has anti-metastasis activity, suggesting that reduced expression of nm23 is associated with the early metastasis (15–20). Interestingly, the increased number of regional lymph node metastases was further shown to be correlated with the poor prognosis of GC (21). The decreased survival rate noted in the malignancy would then suggest a role of lymph node in association with the disease progression. However, the significance of nm23 expression in the primary GC as well as that in the metastatic regional lymph node for monitoring the disease activity and the response to the treatment is not yet known. This study was initiated to address this question. Therefore, in this report, we compared more extensively the difference of nm23 expression between the primary GC and the metastatic regional lymph node in 24 patients with stage III GCC. We also examined a variety of parameters that were associated with the prognosis together with the correlation between these parameters and nm23 expression.

PATIENTS AND METHODS
Tissue Specimens. From January 1984 to December 1995, tissue specimens and 335 regional lymph nodes (range, 4–43 for each patient) from 24 patients with GCC at stage III were collected. Twenty-two patients had total gastrectomy, and two patients had proximal subtotal gastrectomy with a D2 dissection, including N1 (perigastric) and N2 (splenic, left gastric artery, and celiac axis) lymph nodes. Clinical stage of the disease was classified according to the guidelines of the TNM system (22). Patients with lymph node involvement and patients with locoregional recurrence were irradiated with 22 Gy at the afflicted areas. Those with distant metastasis were treated with chemotherapy (2–7). After treatment, all patients were followed routinely. Before surgery, written informed consent was obtained from every patient, and a single-blind procedure was followed to carry out immunohistochemistry and statistic anal-
Fig. 1  Immunohistochemical staining of nm23 expression in primary tumor (A) and metastatic lymph node (B). Original magnification, ×100.
nm23 in Lymph Nodes of Gastric Cancer

RESULTS

The average age of male patients ($n = 20$) was $62.3 \pm 8.3$ years, and that of female patients ($n = 4$) was $65.2 \pm 8.7$ years. Among these 24 patients, 22 were $T_3N_1$, and 2 were $T_3N_2$. In total, 189 metastases were found in 335 dissected lymph nodes. Among these metastatic lymph nodes, $75 (40\%)$ were nm23 positive, and $14 (58\%)$ were nm23 negative (Table 1). On the other hand, in the primary tumor, 15 patients (62.5\%) were nm23 positive, and 9 patients (37.5\%) were nm23 negative (Table 1). However, no difference in age was observed when patients were divided by parameter of nm23 gene expression or clinicopathological factors. There were also no correlations between nm23 gene expression and clinicopathological factors, such as age, sex, tumor size, Borrmann type, differentiation, lymphovascular invasion, and esophageal invasion (data not shown). Correlations between clinicopathological parameters and the survival time following surgery were not remarkable, with the exception of number of metastatic lymph nodes and nm23 expression in the primary tumor (Table 2). Interestingly, correlation between nm23 expression in the primary tumor and the number of metastatic lymph node was highly significant ($\chi^2 = 8.92, P < 0.005$).

When patients were divided by nm23 expression in the primary tumors, a significant difference of survival time was detected (Fig. 2A). The mean survival time of nm23-positive patients was 21.4 months, whereas that of nm23-negative patients was 6 months ($P = 0.0087$). When patients were divided by nm23 expression in the metastatic lymph nodes, the difference between survival was also significant (Fig. 2B). The mean survival time was 22.5 months for nm23-positive patients, and that for nm23-negative patients was 7.0 months ($P = 0.006$). When patients who were nm23 positive in both the primary tumors and the metastatic lymph nodes were grouped together, the difference was more significant (Fig. 2C, $P < 0.005$).

DISCUSSION

For GC, several studies have shown a correlation of clinical response with tumor staging and metastatic lymph nodes (8–

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Table 1 Expression of nm23 in the primary gastric tumor and in the metastatic regional lymph nodes

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex/age (yr)</th>
<th>TNM staging</th>
<th>LN metastasis/ LN dissected</th>
<th>Expression of nm23</th>
<th>Survival time (months)/status</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Primary tumor</td>
<td>Metastatic LN</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M/61</td>
<td>$T_3N_1$</td>
<td>14/39</td>
<td>+</td>
<td>12/dead</td>
</tr>
<tr>
<td>2</td>
<td>M/69</td>
<td>$T_3N_1$</td>
<td>6/19</td>
<td>+</td>
<td>4/dead</td>
</tr>
<tr>
<td>3</td>
<td>M/63</td>
<td>$T_3N_1$</td>
<td>12/19</td>
<td>+</td>
<td>7/dead</td>
</tr>
<tr>
<td>4</td>
<td>M/73</td>
<td>$T_3N_1$</td>
<td>4/4</td>
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<td>8/dead</td>
</tr>
<tr>
<td>5</td>
<td>F/61</td>
<td>$T_3N_1$</td>
<td>3/4</td>
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<td>6/dead</td>
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<td>6</td>
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<td>$T_3N_1$</td>
<td>22/26</td>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>M/56</td>
<td>$T_3N_1$</td>
<td>5/5</td>
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<tr>
<td>9</td>
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</tr>
<tr>
<td>12</td>
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<td>4/6</td>
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<tr>
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<tr>
<td>16</td>
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<td>8/12</td>
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<tr>
<td>17</td>
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<tr>
<td>18</td>
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<tr>
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<td>−</td>
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<td>3/7</td>
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</tr>
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<td>23/dead</td>
</tr>
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<td>12/14</td>
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</tr>
<tr>
<td>24</td>
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<td>2/11</td>
<td>−</td>
<td>4/dead</td>
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</table>

*TNM, tumor-node-metastasis; LN, lymph node; +, positive staining; −, negative staining.
It was shown that the early tumor recurrence and disease progression were associated with rapid growth of tumor cells, evident lymphovascular invasion, and early metastasis to the regional lymph node. Early metastasis of tumor cells to the regional lymph nodes was further suggested to be correlated with the reduced expression of the metastasis suppressor gene \( nm23 \) (15–20, 27).

Recent studies have indicated that \( nm23 \) expression could be associated with G-protein-mediated signal transduction and microtubule assembly, which, in turn, might consummate tumorigenesis and metastasis (28, 29). Tumor cells that had down-regulation of \( nm23 \) gene expression were suggested to be more capable of metastasizing and invading the neighboring tissues (15–20, 27–29). Longer survival was, therefore, anticipated for patients with \( nm23 \) expression in the tumor cells compared to those without (27, 28). Kodera et al. (27) had demonstrated that down-regulation of \( nm23 \)-H1 expression, indeed, correlates with the poor prognosis of patients with human GC. By immunohistochemical staining, Kim et al. (30) further showed that the invasion to the adjacent tissues, lymphatics, lymph nodes, and the distant organs was more likely to be found in the GC patients with decreased \( nm23 \) expression in the primary tumor. However, in a paired normal tumor experiment, Hwang et al. (31) found that expression of \( nm23 \)-H1 mRNA in the primary cancer and metastatic lymph nodes was higher than that in the corresponding normal gastric mucosa. Moreover, without the evident mutation and loss of heterozygosity in the \( nm23 \) gene, they concluded that the expression of \( nm23 \) alone might not warrant the metastasis suppression.

Controversial results between \( nm23 \) expression and prognosis have also been shown in the other cancers, such as breast, colon, and lung cancer (15–20, 27–31). Different results could be due, in part, to the marked heterogeneity of gene expression.
in the tumor cell populations. The nm23 gene may not be expressed constitutively in the cancer cells. By demonstrating the different expression patterns of nm23 in the primary GC and in the metastatic regional lymph nodes, our work suggests that the expression of nm23 in the tumor cells could be affected by the microenvironment. Transfection of nm23 into tumor cells provided another line of evidence for the metastasis suppressor role of nm23 (32); that the metastatic potential of the transfectant was not completely damaged further indicates a potential regulatory process between gene expression and metastasis. These results also correspond well with the known effects of several cytokines, e.g., transforming growth factor-β, interleukin 6, IFN-γ, and tumor necrosis factor-α as well as prostaglandin E2, on nm23 expression (33).

As noted previously, metastasis is a cascade of linked sequential steps involving multiple host-tumor interaction (28, 29). Nonetheless, nm23 expression is different between the primary tumor and the metastatic lymph node, shedding some light on the issue of differential nm23 expression in the various tissues. It is readily conceivable, however, that, besides tumor metastasis, the effect of nm23 expression on the drug sensitivity (34), multidrug resistance, and the other unknown factors that are essential for the tumor cell survival under harsh medical eradication should also be considered. Nevertheless, the detailed biological roles of these factors in the metastasis require further study.

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REFERENCES


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