CD24 Expression in Gastric Adenocarcinoma Is Associated with Tumor Invasiveness

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Background: CD24, also referred to as the heat stable antigen in mice, is a glycosyl phosphatidylinositol-linked glycoprotein expressed by thymocytes, B cells, neutrophils and immature neuronal cells. It has been recently observed in a variety of human malignancy. Here, we demonstrated the expression of CD24 in gastric adenocarcinomas.

Methods: A total of 40 gastric adenocarcinomas and 20 tubular adenomas were immunohistochemically examined for the expression of CD24 and matrix metalloproteinase-2 (MMP-2) proteins. The immunoreactivity of CD24 was semiquantitatively scored (0, 1+, 2+) and compared with clinicopathologic variables and MMP-2 expression in tumor cells.

Results: CD24 was rarely expressed in normal gastric tissue and not expressed in tubular adenoma. In contrast, a moderate/strong expression (2+) of CD24 was observed in 25% of gastric adenocarcinomas, and 30% cases showed a weak CD24 staining (1+). Moreover, CD24 expression was significantly correlated with the depth of tumor invasion and MMP-2 expression.

Conclusions: These results suggest that the aberrant expression of CD24 in gastric adenocarcinomas might be associated with tumor progression and invasiveness.

Key Words: CD24 antigen; Stomach neoplasms; Neoplasm invasiveness; Matrix metalloproteinase-2

Although incidence and mortality rate of gastric cancer have been declined steadily, gastric cancer has been one of the most common malignant neoplasia among both male and female in Korea. In this country, the overall age-standardized incidence rates of gastric cancer were estimated to be 69.6 and 26.8 per 100,000 for males and females, respectively. The prognosis of early gastric cancer (EGC) is usually excellent, and early detection of gastric cancer and improved therapies could lower the mortality rate of gastric cancer during the last decades. However, when the tumor invades beyond the proper muscle layer, overall survival rate is markedly worsen, and thus gastric cancer is still expected that it would be the first leading cause of cancer death through 2005.

CD24, also referred to as the heat stable antigen in mice, is a small heavily glycosylated mucin-like glycoprotein, which is usually expressed in both lymphoid and neuronal cell lineages and involved in the cell adhesion, lymphocyte activation, and apoptosis. In neoplasia, CD24 expression has been described not only in haematologic malignancies, but also in a large variety of solid tumors, such as, breast cancer, prostate cancer, nonsmall cell lung cancer, nasopharyngeal carcinoma, hepatocellular carcinoma, bladder cancer, ovarian cancer, glioma, and neuroblastoma. The physiologic function of CD24 in these cancer cells is not fully elucidated, but there is an evidence suggesting pro-invasive and pro-metastatic roles of CD24 in human tumor cells.

Matrix metalloproteinase-2 (MMP-2) is one of zinc-dependent enzymes that is involved in the degradation of the extracellular matrix. It is especially well known as a decomposing enzymes of type IV collagen, laminin and fibronectin, all of which are components of the basement membrane, and its relationship to the depth of invasion, metastasis in regional lymph nodes, and prognosis of gastric cancer has been reported. In this study, in order to investigate whether there is a relationship between CD24 and MMP-2 expression in gastric adenocarcinomas, immunohistochemical analysis was performed.

Gene expression of CD24 in a human gastric carcinoma cell line, NUHGC-2, was previously reported. To date, however,
very little is known about CD24 in gastric carcinomas and tubular adenomas. Here we investigated the expression of CD24 in our collection of human gastric carcinomas and tubular adenomas by immunohistochemistry and evaluated its association with clinicopathologic variables including MMP-2 expression in cancer cells.

**MATERIALS AND METHODS**

**Tumor sample**

Formalin-fixed and paraffin-embedded specimens were obtained from the Department of Pathology of Chunchun Sacred Heart Hospital, Chunchun, Korea. Tumor specimens included 20 tubular adenomas and 40 gastric adenocarcinomas. Each tumor was classified on the basis of the modified WHO’s classification system and Lauren’s classification.

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissues were cut into 5-μm serial sections, attached to silane-coated slides, deparaffinized in xylene, and rehydrated in phosphate-buffered saline (PBS), pH 7.4. The deparaffinized sections were boiled for 10 min in 0.01 M citrate buffer, pH 6.0, for antigen-retrieval and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 min. Immunohistochemical staining was performed using the EnVision System/HPR (DAKO, Carpinteria, CA, USA). The deparaffinized sections were preincubated with normal goat serum to prevent nonspecific binding, and then incubated overnight at 4 °C with an optimal dilution of the primary antibodies. The sections were rinsed with PBS and incubated with peroxidase-labelled polymer for 30 min at room temperature and then washed with PBS. The enzyme reaction was developed with 0.03% 3,3-diaminobenzidine tetrahydrochloride containing 0.006% hydrogen peroxide. As a negative control, the primary antibodies were replaced by an irrelevant antibody. The primary anti-CD24 antibody (Ab-2, clone 24C02) was purchased from Neomarkers (Fremont, CA, USA), and anti-MMP-2 antibody (clone A-Gel VC2) was purchased from Pharmingen (San Diego, CA, USA).

The staining intensity of CD24 and MMP-2 was scored semi-quantitatively as negative (0), weakly (1+), or moderately/strongly (2+) positive. Negative cases were defined by complete absence of immunoreactivity in the tumor. Any stronger or more extensive staining of the tumor was graded as moderate/strong (2+).

**Statistical analysis**

To compare the expression of CD24 expression with clinicopathologic variables and MMP-2 expression, 3 × 2 contingency tables (e.g., CD24 0/1+/2+ vs Tis-T1 & T2-T4) were set up and z² test was used to determine the strength of association between the investigated variables. p values <0.05 were considered significant. Statistics were calculated with the software package Prism, version 3.0 (GraphPad Software, San Diego, CA, USA).

**RESULTS**

Of the 40 patients with gastric adenocarcinomas, 23 were male and 17 were female. The median age was 63 years (range, 37 to 79 years). Histologically, there were 19 advance gastric carcinomas (AGCs) and 21 EGCs. The clinicopathologic features of the cases are summarized in Table 1. In addition, 20 cases of tubular adenoma (patients’ age 49-76, median 65) were included in this study to compare the CD24 expression in gastric tubular adenomas and adenocarcinomas.

**Table 1. Relationship between CD24 expression and clinicopathologic variables**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of all cases</th>
<th>CD24 expression</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Carcinomas</td>
<td>40</td>
<td>18 (45.0)*</td>
<td>12 (30.0)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>8 (20.0)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>10 (25.0)</td>
<td>8 (20.0)</td>
</tr>
<tr>
<td>Age at surgery (year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>24</td>
<td>11 (27.5)</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>≥65</td>
<td>16</td>
<td>7 (17.5)</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Invasion depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa &amp; submucosa</td>
<td>21</td>
<td>11 (27.5)</td>
<td>9 (22.5)</td>
</tr>
<tr>
<td>Muscle or more</td>
<td>19</td>
<td>7 (17.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>LN metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
<td>14 (35.0)</td>
<td>9 (22.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>4 (10.0)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Lauren’s classification</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intestinal</td>
<td>20</td>
<td>8 (20.0)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>1 (2.5)</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>15</td>
<td>9 (22.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>MMP-2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>13 (32.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>5 (12.5)</td>
<td>9 (22.5)</td>
</tr>
</tbody>
</table>

*p Parenthesis indicates the percentage.*
To detect the CD24 expression in non-neoplastic and neoplastic tissues, formalin-fixed, paraffin-embedded tissue sections were stained with Ab-2 antibody, which had been known to be useful for the detection of CD24 expression in paraffin-embedded tissues. In normal gastric tissue, CD24 was rarely expressed, only focally cytoplasmic staining of mucosal epithelial cells (Fig. 1A). Tubular adenomas displayed no immunoreactivity against CD24 (Fig. 1B). In contrast, we observed CD24 expression in 55% of gastric adenocarcinomas: 12 cases (30%) showed a weak CD24 immunopositivity and 10 cases (25%) showed a moderate/strong immunopositivity. The staining quality was either cytoplasmic (Fig. 1C), membranous (Fig. 1D), or both. Generally, CD24 immunostaining showed considerable intratumoral heterogeneity.

In invasive carcinomas, we did not find any significant association of overall CD24 expression with patient’s gender and age, and histologic types of tumors (Table 1). However, CD24 expression was significantly correlated to the invasion depth of gastric carcinoma (p=0.006, Table 1). CD24 was not expressed in about a half of EGC cases, and most of CD24-positive EGCs showed only weak staining. In AGC, about a half of cases displayed moderate or strong immunoreactivity and 3 cases (16%) showed weak staining. Furthermore, statistically significant correlation between CD24 positivity in gastric adenocarcinomas and lymph node metastasis was also found (p=0.027; Table 2). However, lymph node metastasis was not detected in any cases of EGC included in this study, and CD24 immunoreactivity only in AGC was not significantly correlated with lymph node metastasis of tumor cells (data not shown).

In 40 cases of gastric carcinoma included in this study, 21 cases (53%) showed immunoreactivity for MMP-2 in the carcinoma cells (Fig. 2). MMP-2 immunoreactivity was detected in 16 (73%) of 22 CD24-positive adenocarcinomas, while only 5 (28%) of 18 CD24-negative cases showed MMP-2 immunoreactivity, implying the statistically significant association between CD24 and...
MMP-2 expression in gastric carcinoma cells \( (p=0.018; \text{Table 1}) \). When the analysis for EGCs and AGCs was repeated to access whether the depth of tumor invasion affect the association of MMP-2 expression with staining intensity of CD24 in gastric adenocarcinomas, significances was only found in EGCs (EGCs, \( p=0.004 \); AGCs, \( p=0.917 \)). In contrast, the association of CD24 immunoreactivity with the invasion depth of tumors was independent of the status of MMP-2 expression in tumor cells, as CD24 positivity of the tumor significantly was correlated to the invasion depth in both MMP-2-negative and -positive groups (MMP-2-negative tumor, \( p=0.045 \); MMP-positive tumor, \( p=0.019 \)). MMP-2 was not expressed in any of the normal gastric epithelium studied.

**DISCUSSION**

In this study, tissues of gastric adenocarcinomas and tubular adenomas were immunohistochemically examined for the expression of CD24 protein. We found CD24 expression in 55% of gastric adenocarcinomas but not in any cases of tubular adenomas. Moreover, CD24 expression was significantly correlated with the invasion depth of tumors and MMP-2 expression. Previous studies have demonstrated CD24 expression in a wide variety of human malignancies. On tumor cells, CD24 has attracted interest as a ligand for P-selectin, P-selectin, expressed by activated platelets and endothelial cells, binds to ligands on myeloid cells and subsets of lymphocytes. Recent studies have provided evidence that certain tumor cells can also bind to platelets and endothelial cells via CD24-P-selectin interaction, raising the possibility that CD24 expressing tumor cells can disseminate more easily due to their capacity to form thrombi with activated platelets, or to adhere to endothelia in the bloodstream. In addition, the correlation between CD24 expression and invasiveness had been reported in mammary carcinoma cell lines and bladder transitional carcinoma tissues, and over expression of CD24 in glioma cell line stimulated the migration and invasion of tumor cells. In this study, we found CD24 immunoreactivity in gastric adenocarcinomas but not in tubular adenomas. Interestingly, tumors that invaded beyond submucosa showed a higher rate of CD24 positivity on tumor cells, supporting the previous reports showing the correlation between CD24 expression and tumor invasiveness. CD24 expression in EGCs was also significantly correlated with MMP-2 expression, which has been reported to be related with the depth of invasion, metastasis in regional lymph nodes, and prognosis of gastric cancer. However, the number of cases \( (n=21) \) was insufficient for valid evaluation of the association of MMP-2 expression with CD24 immunoreactivity, and the significance was not found in AGCs.

In addition to invasion depth, lymph node metastasis is one of the most important factors that contribute to the prognosis and survival of gastric cancer patients. In this study, CD24 expression was significantly correlated with lymph node metastasis, when both EGCs and AGCs are included in statistical analysis, while its correlation was not found in AGC only, suggesting CD24...
expression did not seem to be an independent factor associated with lymph node metastasis of gastric carcinoma. A limitation of our study in comparing the CD24 expression and lymph node metastasis of tumor cells are small number of AGC cases without lymph metastasis. This study was designed to investigate whether CD24 antigen is expressed on gastric adenocarcinomas and tubular adenomas. Thus, only limited numbers of cases are included in this study and it may be impossible on the ground of our data to decide whether the CD24 expression on gastric adenocarcinomas is correlated with the lymph node metastasis of tumor cells or not.

In conclusion, our findings suggest that the CD24 expression in gastric adenocarcinomas may be associated with tumor progression and invasiveness.

REFERENCES


