Immunohistochemical Study on the Expression of Cyclin D1 and E in Gastric Cancer

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Summary: Cyclin D1 and E have been found to be deregulated and overexpressed in various types of cancers. In order to study the cell cycle regulatory mechanisms in gastric cancer, we have analyzed the protein expression of cyclin D1 and cyclin E in 76 tumor specimens from patients with primary gastric cancer, using immunohistochemistry. Overexpression of cyclin D1 was observed in 38 cases (50.0%). Overexpression of cyclin E was observed in 40 cases (52.6%). There was no significant difference between the expression of cyclin D1 and any clinicopathological factor. Cyclin E overexpression was correlated with a high incidence of lymph node metastasis, a low incidence of T1, and with Stage I. There was no significant difference in survival curves between cyclin D1 (+) and cyclin D1 (−). The survival curves of cyclin E (−) were significantly higher than those of cyclin E (+). These results suggested that in gastric carcinoma, cyclin E overexpression was useful as a prognostic indicator, but cyclin D1 was not.

Key words cyclin D1, cyclin E, gastric cancer, immunohistochemistry

INTRODUCTION

Recent genetic analyses have revealed abnormalities in cell-cycle regulators, which are likely to contribute to carcinogenesis via aberrant cell-cycle progression. A restriction point has been identified late in the G1 phase, just before the G1/S phase transition, where cycling is stimulated by G1 phase cyclins, perhaps the key molecules in cell-cycle control [1]. Cyclin D1 and Cyclin E belong to the family of G1 cyclins, and constitute a critical target for proliferative signals in G1 [2,3]. The p53 gene product has been proposed as a cell-cycle regulator at the G1/S phase checkpoint, where in-vitro experiments using cancer cell lines have indicated that cyclin D1 and cyclin E may be involved in p53-mediated growth suppression [4,5]. Passage through the G1/S phase is based on the regulation activity of a family of related protein kinases (cyclin-dependent kinases or Cdks) [6]. Cyclins have been demonstrated to promote cell cycle transitions by binding and activating specific Cdks [7,8]. Progression from G1 to S phase in mammalian cells involves activation of cyclin E/Cdk2, cyclin D/Cdk4, and cyclin A/Cdk2 [6]. Cyclins D1 and E accumulate and activate distinct Cdk proteins in G1, while the kinetics of cyclin A accumulation and Cdk activation suggest an S-phase role [6]. Cyclins D1 and E overexpression have been reported in several types of human cancer [9-15]. In the present study, we examined the significance of the expression of cyclin D1 and of cyclin E in surgical specimens of gastric carcinoma.

MATERIALS AND METHODS

Tissues

Samples of gastric carcinoma were taken from the stomach of 76 patients who had undergone gastrectomy for gastric carcinoma. The number of those at Stage I, Stage II, Stage III, and at Stage IV was 20,
Samples were obtained from the central zone of the carcinoma lesion and an immediately-fresh-frozen section of each sample, fixed in freshly-prepared 4% paraformaldehyde and stored at −80 °C until examined by immunohistochemistry.

Immunohistochemistry

Seventy-six sections were stained using the avidin-biotin-peroxidase technique (ABC method). In brief, after frozen sections were washed by PBS, sections were fixed in cold acetone for 10 min, washed by PBS again, and then immersed in methanol containing 0.3% H$_2$O$_2$ for 30 min to block endogenous peroxidase activity. The sections were then incubated with anti-cyclin D1 monoclonal antibody (diluted 1:500 Novocastra Laboratories, Newcastle, UK) or anti-cyclin E monoclonal antibody (diluted 1:500 Pharmingen, San Diego, CA), for at least 12 hrs at 4 °C followed by incubation with biotinylated rabbit anti-mouse serum for 30 min and incubation with streptavidin-peroxidase complex for 30 min. Staining was developed by incubating the sections in 3-amino-9-ethylcarbazole (AEC) for 5 min. The sections were then counterstained in hematoxylin, and mounted. Nonimmuno serum was used for the negative control for each staining. The intensity of the immunohistochemical reaction was evaluated in at least ten random microscopic high power fields at 400× magnification. Less than 10% positive cells was scored as negative (−), while more than 10% positive cells was scored as positive (+).

Statistical analysis

The χ$^2$ test was used for comparing group frequencies, as appropriate. P < 0.1 by the two-tailed test was considered statistically significant. Survival curves were computed using the Kaplan-Meier method, and differences between survival curves were compared using the Cox-Mantel test. Differences were considered significant at the 5% level.

RESULTS

Immunohistochemical staining

Positive staining of cyclin D1 and of cyclin E protein were recognized in the nucleus of cancer cells (Figs 1a and b). Overexpression of cyclin D1 was observed in 38 cases (50.0%). Overexpression of cyclin E was observed in 40 cases (52.6%).

The expression of cyclin D1, cyclin E and clinicopathological factors

The relation between the expression of cyclin D1, cyclin E and the clinicopathological factors including H, P, N, T factors and stage of the gastric carcinoma are shown in Table 1. There was no significant difference between the expression of cyclin D1 and any clinicopathological factor (Table 1). Cyclin E overexpression was correlated with a high incidence of...
TABLE 1.
Clinicopathological features, cyclin D1 and E immunoreactivities in gastric carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Cyclin D1 (%)</th>
<th>Cyclin E (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>1 (2.6)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>P</td>
<td>4 (10.5)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>LN</td>
<td>27 (71.7)</td>
<td>24 (63.2)</td>
</tr>
<tr>
<td>T1</td>
<td>4 (10.5)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>T2</td>
<td>7 (18.4)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>T3</td>
<td>22 (57.9)</td>
<td>20 (52.6)</td>
</tr>
<tr>
<td>T4</td>
<td>5 (13.2)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>St. I</td>
<td>8 (21.1)</td>
<td>12 (31.6)</td>
</tr>
<tr>
<td>St. II</td>
<td>4 (10.5)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>St. III</td>
<td>15 (39.5)</td>
<td>12 (31.6)</td>
</tr>
<tr>
<td>St. IV</td>
<td>11 (28.9)</td>
<td>11 (28.9)</td>
</tr>
</tbody>
</table>

*p<0.1, **p<0.05
H: liver metastasis; P: peritoneal dissemination; Ly: lymph node metastasis; T1: mucosa or submucosa; T2: muscularis propria or subserosa; T3: penetrates serosa; T4: adjacent structures; St.: stage

lymph node metastasis (77.5% vs 55.6%; p<0.1), a low incidence of T1, and with Stage I (7.5% vs 30.6%, and 15.0% vs 38.9%, respectively; p<0.05) (Table 1).

The expression of cyclin D1, cyclin E and prognosis

The 1-year, 3-year, and 5-year survival rate of cyclin D1 (+) cases was 54.1%, 38.0%, and 30.4%, respectively. The 1-year, 3-year, and 5-year survival rate of cyclin D1 (-) cases was 77.7%, 55.7% and 44.6%, respectively. The 1-year, 3-year, and 5-year survival rate of Stage III of cyclin D1 (+) cases was 53.3%, and 33.3%, and 0%, respectively. The 1-year, 3-year, and 5-year survival rate of Stage III of cyclin D1 (-) cases was 72.7%, 58.2%, and 58.2%, respectively. There was no significant difference in survival curves between cyclin D1 (+) and cyclin D1 (-) in both all stages, and in Stage III cases (Figs 2a and b, respectively). On the other hand, the 1-year, 3-year, and 5-year survival rate of cyclin E (+) cases was 55.3%, 32.0%, and 16.0%, respectively. The 1-year, 3-year, and 5-year survival rate of cyclin E (-) cases was 79.9%, 65.9%, and 56.4%, respectively. The 1-year, 3-year, and 5-year survival rate of Stage III of cyclin E (+) cases was 60.0%, 30.0%, and 0%, respectively. The 1-year, 3-year, and 5-year survival rate of Stage III of cyclin E (-) cases was 81.8%, 72.7%, and 60.6%, respectively. The survival curves of cyclin E (-) were significantly higher than those of cyclin E (+) in both all stages, and in Stage III cases (p<0.01, p<0.05, respectively) (Figs 3a and b, respectively).

Fig. 2. a: Survival rate according to the expression of cyclin D1.
b: Survival rate in Stage III according to the expression of cyclin D1.
DISCUSSION

In human colorectal, esophageal and gallbladder cancers, overexpression of cyclin D1 is an early event in carcinogenesis [9,10,12]. In human esophageal, breast, urinary, and gallbladder cancer, cyclin D1 overexpression has been useful as a prognostic indicator associated with a poor prognosis [9-11,16]. Itoi et al. [12] reported that lymphatic permeation, venous permeation, and lymph node metastasis were frequent in specimens of gallbladder carcinoma showing cyclin D1 overexpression. However, in our study, there was no significant difference between the expression of cyclin D1 and any clinicopathological factor, and no significant difference in survival curves between cyclin D1 (+) and cyclin D1 (−) cases in specimens of gastric carcinoma, either. Youssef et al. [17] reported that lesions showing cyclin D1 overexpression were more likely than lesions without such overexpression to have a high degree of proliferating cell nuclear antigen (PCNA) positivity. In contrast, we have already reported that no significant correlation was seen between cyclin D1 overexpression and PCNA Labelling Index (LI) in gastric carcinomas [18]. Lee et al. [11] found that the expression of cyclin D1 and p53 showed an inverse correlation to grade in urinary carcinomas. But we have already reported that cyclin D1 was not correlated with p53 overexpression in gastric carcinomas [18]. Different pathways involving cyclin D1 and p53 may be active in the carcinogenesis in the urinary bladder, and in the stomach. In human breast, urinary, and gastric cancer, cyclin E overexpression has been useful as a prognostic indicator associated with a poor prognosis [13-15,19]. Akama et al. [13] have reported that all cases with cyclin E gene amplification displayed lymph node metastasis in gastric carcinoma. Sakaguchi et al. [20] reported that strong cyclin E expression was frequently observed in deeply invasive tumors, tumors with lymph node metastasis, and in tumors at advanced stage, in gastric carcinoma. In our study, cyclin E overexpression was correlated with a high incidence of lymph node metastasis, a low incidence of T1, and with Stage I, and the survival curves of cyclin E (−) were significantly higher than those of cyclin E (+). Yasui reported [21] that the expression of cyclin E was correlated with proliferating activity and with p53 status in gastric carcinomas. We have already reported that cyclin E was correlated with PCNA LI and with p53 overexpression in gastric carcinomas [18].

In conclusion, the present study indicated that in gastric carcinoma, cyclin E overexpression was useful as a prognostic indicator, but cyclin D1 was not.

REFERENCES

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EXPRESSION OF CYCLIN D1 AND E


