Cathepsin E and Subtypes of Intestinal Metaplasia in Carcinogenesis of the Human Stomach

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Key Words

- cathepsin E;
- gastric cancer;
- gastric dysplasia;
- intestinal metaplasia

Background. Cathepsin E is found mainly over the gastric surface and foveolar epithelial cells, and it also is found in the metaplastic pyloric glands and cancer cells. The exact function of cathepsin E in gastric mucosa remains unclear. The colonic type (type III) of intestinal metaplasia (IM) is strongly associated with intestinal-type gastric carcinoma. IM is considered to be a precancerous lesion. The aim of this study was to find out the role of cathepsin E in IM, dysplasia, and cancer of stomach.

Methods. Sixty-nine biopsy specimens with IM and dysplasia and 33 gastrectomy specimens with gastric cancer were fixed, sectioned, and stained with PAS-alcian blue stain, high iron-diamine alcian blue stain to classify IM and immunohistochemical stain to localize cathepsin E. Those patients with dysplastic gastric lesions received regular endoscopic follow-up.

Results. Fifteen of 69 patients with gastric dysplasia developed cancer in a median 10.5 months follow-up. Severe dysplasia developed cancer significantly higher than mild dysplasia (12/20 vs. 1/25, p < 0.001), and type III in intestinal metaplasia seemed to have significantly prevalent differentiation for severe dysplasia and gastric cancer. Cathepsin E was stained in intestinal metaplasia with dysplastic change in 44/69 specimens (63.8%), and cancer in 28/48 (58.3%) specimens, there was no significant difference between intestinal type and diffuse type carcinoma in cathepsin E staining. The positive staining for cathepsin E decreased significantly in severe dysplastic gastric mucosa.

Conclusions. Type III IM is commonly associated with severe dysplasia and cancer, and it may be a precursor lesion. The positive staining of cathepsin E decreased with the severity of gastric dysplasia, representing dedifferentiation of the cells.

Epidemiological and morphological studies have shown that there is an association between intestinal metaplasia (IM) and gastric cancer. Progressively changes from chronic atrophic gastric tissue to IM, and cancer were described before. Three variants of IM have been identified. Several authors have found that sulfomucin-secreting IM sub type (type III or “colonic” type) is strongly associated with intestinal cancer.

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nal-type gastric carcinoma, while the risk of cancer is low in the other two subtypes. The human gastric mucosa contains two types of cathepsins, cathepsin D and cathepsin E (previously called slow-moving proteinase, SMP). Cathepsin E is found mainly in the gastric surface and foveolar epithelial cells, and in some reticuloendothelial cells, whereas cathepsin D is found in mucous neck cells, parietal cells, pyloric gland cells and macrophages. Besides normal cells, some cancer cells were also found to have proteinase. Cathepsin E is the most common proteinase produced by gastric cancers. Dysplastic lesion in the stomach may result from inflammatory reaction or malignant transformation. Malignant change was found in 60% of severe dysplastic lesions during a 5-year follow-up. How ever, the different expression of cathepsin E in dysplasia gastric mucosa accompanied with IM gastric mucosa has not been depicted well. The purpose of this study was to use immunohistochemical method to localize cathepsin E in the human stomach with malignant or premalignant lesions.

**Methods**

From 1990-9 to 1995-5, gastrectomy specimens from 33 patients with gastric carcinoma were fixed in 10% formalin, sectioned, and embedded in paraffin. The histological type of each cancer was determined according to the criteria of Lauren. Sixty-nine patients with endoscopic biopsy-proven IM and dysplasia were also included. Multiple gastric biopsies, whenever possible, were taken from the lesions. All specimens were fixed in 10% formalin and routinely embedded in paraffin. Serial sections were cut for (1) hematoxylin-eosin stain, (2) alcian blue pH 2.5-9 periodic acid-Schiff (PAS/AB) and high iron-diamine plus alcian blue pH 2.5 (HID/AB) stain to identify neutral, sialomucin and sulfomucins, and (3) immunohistochemical stain to localize cathepsin E. IM was characterized by the presence of goblet cells secreting prominent sulfomucins, columnar mucous cells secreting non-sulfated mucins and absence of Paneth cells. Type III (complete) IM resemble type II IM in most respects, but the columnar mucous cells secrete pre dominantly sulfomucins.

**Immunohistochemical staining**

Aspartic proteinases were identified by peroxidase anti-peroxidase (PAP) stain ing technique (Dako PAP Kit, System 40, Dako Co oper a tion, Carpinteria, California). The primary antibodies for cathepsin E were raised in New Zealand rabbits as described previously. Sections (5 µm) were deparaffinized in xylene, rehydrated in decreasing concentration of alcohol and then rinsed in water. The specimens were exposed in 3% hydrogen peroxide to destroy the endogenous peroxidase activity, rinsed with water, and washed in 0.05 tris-buffer saline (TBS) for 10 minutes. These specimens were incubated in normal swine serum for 20 minutes and were incubated in 1:1000 primary antibodies (anti-SMP) overnight at room temperature. After a 20-minute wash in 0.05 M TBS and one-hour incubation in swine anti rabbit immunoglobulin, the specimens were washed again in TBS. After incubation in soluble horse radish peroxidase-rabbit anti-horseradish peroxidase (PAP) complex for 20 minutes and wash ing in TBS, speci mens were incubated in substrate solution containing 3-amino-9-ethylcarbazole in N, N-dimethylformamide and 0.3% hydrogen peroxide for 15-40 minutes. They were then washed in water and counter stained with Meyer’s hematoxylin and mounted with glycerin-gelatin (Merck). Those cells stained red with clear background were defined as positive staining.

Gastric dysplasia was defined as prominent cellular and structural abnormalities of gastric epithelium. They were graded as mild, moderate, and severe degree according to the method of Morson et al. and to the definition of Ming et al. which are accepted by the World Health Organization. With increasing degree of dysplasia, the nuclei tend to vary in size, to appear irregular, and to contain prominent nucleoli. In addition, the nuclei are more prominent and mitotic ac-
tivity increase. Cell stratification, loss of polarity, and disorganization of gland architecture develops. The cathepsin E staining was also graded as grade 1 (less than 1/2 cells with positive staining), grade 2 (1/2 to 2/3 cells with positive staining) and grade 3 (more than 2/3 of cells with positive staining) according to the extent of positive staining involved. (Fig. 1, 2, and 3).

All the patients with gastric dysplasia were regularly followed with endoscopic biopsy until completely healing of gastric lesion or positive testing for malignancies. Repeated endoscopic biopsy in severe dysplasia patients was adopted. If the biopsied specimens raised the suspicion of malignancy, surgical intervention was suggested. In those patients with moderate dysplasia, follow-up endoscopic biopsy after two months of treatment were arranged. If the grades of dysplasia became severe, treatment was adopted as severe dysplasia protocol. In those patients with mild dysplasia, regular follow-up was arranged every three to six months.

Statistical analysis was performed using the chi-square and Fisher’s exact tests. The significant level was \( p < 0.05 \).

Among the 69 patients with gastric dysplasia, 15 patients were found to have malignant change after a median 10.5 (range 1-23.5) months follow-up. Patients’ characteristics of gastric dysplasia are shown in Table 1. Dysplastic or cancerous lesions were more common in mucosa of antrum and angularis but less common in fundus and cardia. As association between the types of IM and grades of dysplasia is shown in Table 2. There was significant association between IM and dysplasia (\( p < 0.001 \)). Type III IM seemed to have significant predilections for severe dysplasia (\( p < 0.001 \)). Malignant change was more common in severe dysplasia than mild dysplasia (12/20 vs. 1/25, \( p < 0.001 \)). Among the 28 cases of intestinal-type gastric carcinoma, 18 cases were associated with positive cathepsin E staining (64.3%) whereas 16 diffuse-type gastric carcinoma had 8 cases (50%) as so ciated with positive cathepsin E staining (\( p > 0.05 \)). However, a

**Results**

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**Fig. 1.** Grade I positive staining cathepsin E in intestinal metaplasia mucosa which shows less than 1/2 cells with positive staining.

**Fig. 2.** Grade 2 positive staining cathepsin E in intestinal metaplasia mucosa which shows between 1/2 to 2/3 cells with positive staining.

**Fig. 3.** Grade 3 positive staining cathepsin E in gastric cancer cells which shows more than 2/3 of cells with positive staining.
trend to decrease in cathepsin E staining was found in severe dysplastic change of gastric mucosa ($p < 0.001$) (Table 3).

### Discussion

IM is a common associated finding in chronic gastritis, ulcer and cancer. The prevalence rate in gastric biopsy specimen was about 20-37% in previous study and was relatively more common in antral mucosa and less common in body mucosa.\textsuperscript{16-18} In our study, type I IM has been found to be more frequently associated with benign gastric conditions, whereas type III IM was of ten present in severe dysplasia, particularly in cases with malignant transfor mation. Although none of the patients with type I IM and gastric dysplasia developed cancer during the follow-up period, 5 patients with gastric cancer associated with type I IM. All three subtypes of IM may be associated with malignant transformation, but subtype III IM played a more important role than the other two subtypes. Many authors consider dysplasia as a precancerous lesion.\textsuperscript{8,19} Our study shows that malignant change was found in 21.7% of patients with dysplasia, and the malignant transformation in increase with the grade of dysplasia (60% in severe dysplasia versus 4% in mild dysplasia, and 8.2% in moderate dysplasia). In the 11 patients with severe gastric dysplasia associated with type III IM, 10 patients (91%) developed gastric cancer later. Therefore, those specimens combining severe dysplasia and type III IM may have increased malignancy potential; close follow-up and early surgical intervention is mandatory.

The exact physiological role of cathepsin E in the stomach is not clearly understood. Cathepsin E is present in tissues other than gastric mucosa, such as lymphoid fol li cles and spleen, bone marrow. It may play a role in immunological conditions.\textsuperscript{20,21} Using antiserum against cathepsin E purified from the rat spleen and human erythroid cells and spleen, Saku et al. found that cathepsin E was absent in pre cancerous lesions and well-differentiated adenocarcinoma.\textsuperscript{22} In contrast to the Saku study, we found that cathepsin E was present in 63.8% of dysplastic gastric mucosa, 64.3% of intestinal-type gastric carcinoma and 50% of diffuse-type gastric cancer. There was no difference between the intestinal and diffuse types of gastric cancers in our patients. How ever, a trend to disappear in cathepsin E staining was found in our patients.

### Table 1. Patients' characteristics in gastric dysplasia and cancer

<table>
<thead>
<tr>
<th></th>
<th>Dysplasia (n=69)</th>
<th>Gastric cancer (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>67/2</td>
<td>32/1</td>
</tr>
<tr>
<td>Age (mean ± SD, yrs)</td>
<td>70.2 ± 8.7</td>
<td>72.5 ± 10.2</td>
</tr>
<tr>
<td><strong>Locations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardia</td>
<td>3 (1)</td>
<td>2</td>
</tr>
<tr>
<td>Fundus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Body</td>
<td>15 (4)</td>
<td>10</td>
</tr>
<tr>
<td>Angle</td>
<td>24 (3)</td>
<td>9</td>
</tr>
<tr>
<td>Antrum</td>
<td>26 (7)</td>
<td>10</td>
</tr>
<tr>
<td><strong>Type of lesions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer/erosion</td>
<td>58 (11)</td>
<td>19</td>
</tr>
<tr>
<td>Mass/polyps</td>
<td>4 (2)</td>
<td>7</td>
</tr>
<tr>
<td>Nodules</td>
<td>2 (2)</td>
<td>7</td>
</tr>
<tr>
<td>Gastritis</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

n = No. of patients with malignant change.

### Table 2. Subtypes of intestinal metaplasia and different grades of dysplasia

<table>
<thead>
<tr>
<th>Dysplasia grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>19</td>
<td>5</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>6</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>18</td>
<td>15</td>
<td>69</td>
</tr>
</tbody>
</table>

\(p < 0.001\); \(b p < 0.001\).

### Table 3. Immunohistochemical Staining for cathepsin E in 48 patients with gastric cancer and 69 patients with dysplasia

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer (n=48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28 (58.3%)</td>
</tr>
<tr>
<td>Intestinal (n=28)</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>18 (64.3%)</td>
</tr>
<tr>
<td>Diffuse (n=16)</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Unclassified (n=4)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Dysplasia (n=69)</td>
<td>21</td>
<td>16</td>
<td>7</td>
<td>44</td>
<td>63.8%</td>
</tr>
<tr>
<td>Mild (n=25)</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>22</td>
<td>88%</td>
</tr>
<tr>
<td>Moderate (n=24)</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>15</td>
<td>66.7%</td>
</tr>
<tr>
<td>Severe (n=20)</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>35%</td>
</tr>
</tbody>
</table>

\(a\) No. of cases.
\(b p < 0.001\), Chi-square for trend.
with severe dysplastic gastric mucosa. Depletion of enyzmotic activity of acid pro tease has been shown in gastric carcinoma by Khn and Bezuidenhout. Absence of cathepsin E staining in gastric tissue may represent a trend for dedifferentiation of cells during malignant transformation. Close fol low-up and early surgery is mandatory.

\[ \text{Pathology 1979;3:191-9.} \]


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Lauren P. The two histological main types of gas tric car ci na: dif fuse and so-called in tes ti nal-type car ci na: a turn ment at a histo-clinical clas si fi ca tion *Acta Pathol Microbiol Scand* 1965;64:31-49.


Khn SH, Bezuidenhout DDJ. En zy matic char ac ter i za tion and immu no histo chem ical lo cal iza tion in gas tric mucosa. *Gastroenterology* 1987;93:77-84.

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6. Jass JR, Filipe MI. A vari ant of in tes ti nal meta plut asia as so ci ated with gas tric car ci no ma: a histo che mi cal study. *Histo -