The Expression of Cathepsin D in Uterine Cervical Intraepithelial Neoplasia Correlates with the Nuclear Expression of Ki-67 and p53

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Abstract: The lysosomal protease cathepsin D is associated with tumor progression in malignant tumors, but its presence in pre-malignant dysplastic cells has not been established. The purpose of this study is to evaluate the expression patterns of the cathepsin D, Ki-67, and p53 proteins in dysplastic cells in uterine cervical intraepithelial neoplasias (CINs). In 52 patients with uterine CINs, expression of the cathepsin D, Ki-67, and p53 proteins was assessed in dysplastic cells by immunohistochemistry with monoclonal antibodies against cathepsin D, Ki-67, and p53. Immunohistochemical analysis revealed high levels of cathepsin D (>10% cathepsin D-positive dysplastic cells) in 3 of 17 cervical intraepithelial neoplasia (CIN) 1 specimens, 16 of 20 CIN2 specimens, and in all 15 CIN3 specimens. Cathepsin D expression was significantly higher in CIN2 and CIN3 specimens than in CIN1 specimens (P<0.01), but did not differ significantly between CIN2 and CIN3 specimens. The proportion of dysplastic cells that expressed nuclear Ki-67 was 26.41% in CIN1 specimens, 50.35% in CIN2 specimens, and 76.33% in CIN3 specimens. Ki-67-positive dysplastic cells were significantly increased in CIN2 and CIN3 specimens as compared to CIN1 specimens (P<0.01), and in CIN3 specimens as compared to CIN2 specimens (P<0.01). High expression of nuclear p53 (>10% p53-positive dysplastic cells) was detected in 4 of 17 CIN1 specimens, 12 of 20 CIN2 specimens, and in 11 of 15 CIN3 specimens. Nuclear p53 expression was significantly higher in CIN2 and CIN3 specimens than in CIN1 specimens (P<0.01), but did not differ significantly between CIN2 and CIN3 specimens. Compared to dysplastic cells in CIN1, dysplastic cells in CIN2 have an increased invasive growth potential, as shown by their increased expression of cathepsin D, a higher proliferating potential as shown by their increased expression of Ki-67, and a higher p53 expression.

Key words: cathepsin D, uterine CIN, immunohistochemistry, Ki-67, p53
Introduction

Malignant tumor cells invade the surrounding matrix and penetrate the basement membrane. This process is a fundamental characteristic of cancer cells. Proteases are thought to play an important role in tumor invasion and metastasis because they degrade extracellular matrices and basement membranes\(^1,2\). An extracellular matrix consists of multiple components, which include collagens, glycoproteins, and proteoglycans. Thus, extracellular matrix degradation must involve a complex family of proteases.

Cathepsin D is a lysosomal acidic protease that was first identified as a 52 kDa estrogen-dependent glycoprotein in MCF-7 cells. Cathepsin D activates cathepsin B and urokinase-type plasminogen activator, and these enzymes initiate the proteolytic cascade that may be responsible for the breakdown of basement membrane proteins\(^3\). Thus, cathepsin D is thought to be the key enzyme in this process, and it has attracted considerable attention because of its potential role in tumor invasion and metastasis.

The clinical importance of cathepsin D expression has been discussed in the context of various tumors. High expression of cathepsin D correlates closely with poor prognosis and tumor progression in breast cancer\(^4,7\), colorectal cancer\(^8\), and thyroid cancer\(^9\). Whether cathepsin D is expressed in pre-malignant dysplastic cells is still unclear.

To define the characteristics of dysplastic cells in uterine cervical intraepithelial neoplasia (CIN), we have correlated their potential for invasive growth assessed as the expression of cathepsin D with their proliferating potential assessed by Ki-67 expression and their over-expression of p53, at each histological grade.

Methods

Specimens

Tissues obtained from 52 patients with uterine CIN who had undergone biopsy were examined in the First Department of Pathology, Showa University. Of the 52 specimens, 17 were identified as CIN1 (Fig. 1a; age range of the patients: 19–56 years, mean ± SD = 37.5 ± 9.1), 20 as CIN2 (Fig. 2a; age range of the patients: 19–57 years, mean ± SD = 40.3 ± 13.1), and 15 as CIN3 (Fig. 3a; age range of the patients: 29–45 years, mean ± SD = 37.5 ± 8.3). There was no significant difference in age among the patients.

Immunohistochemical evaluation of the specimens

Specimens were routinely fixed in 10% buffered formalin and embedded in paraffin wax. Four consecutive paraffin wax-embedded sections (4-μm thick), containing dysplastic and non-dysplastic epithelium, were prepared for immunostaining. The sections were dewaxed using xylene and transferred to alcohol. The slides were then placed in citric acid buffer (10 mM) and heated in a microwave oven (700 W) for 12 minutes to expose the antigens. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide in methanol for 30 minutes. Slides were then washed three times in phosphate-buffered saline (PBS) and incubated in 10% normal goat serum for 20 minutes to reduce non-specific antibody binding. After washing with PBS, three slides from each specimen were incubated overnight at 4 °C with monoclonal anti-cathepsin D antibody (H908, Nichirei, Tokyo, Japan), monoclonal anti-Ki-67 antibody (A0047, DAKO, Kyoto, Japan), or monoclonal anti-p53 antibody (DO7, Novocastra, Newcastle, UK). The slides were washed with PBS and incubated for 30 minutes with biotinylated anti-mouse immunoglobulin antibody (Histofine ABC Kit;
Cathepsin D expression in uterine CIN

Fig. 1.

Fig. 2.
Table 1. Immunohistochemical analysis of dysplastic cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CIN1 (n = 17)</th>
<th>CIN2 (n = 20)</th>
<th>CIN3 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD (positive : negative)</td>
<td>3 : 14*</td>
<td>16 : 4</td>
<td>15 : 0</td>
</tr>
<tr>
<td>Ki-67 LI (% )</td>
<td>26.41 ± 12.16*</td>
<td>50.35 ± 18.12**</td>
<td>76.33 ± 18.55</td>
</tr>
<tr>
<td>p53 (positive : negative)</td>
<td>4 : 13*</td>
<td>12 : 8</td>
<td>11 : 4</td>
</tr>
</tbody>
</table>

CD : cathepsin D
Ki-67 LI : Ki-67 labeling index
*P < 0.01, CIN1 vs CIN2 and CIN3
**P < 0.01, CIN2 vs CIN3

Fig. 1. Histopathological and immunohistochemical analysis of CIN1.
a : HE staining. b : Immunostaining with anti-cathepsin D antibody shows no reactivity. c : Immunostaining with anti-Ki-67 antibody has a labeling index of 28.57%. d : Immunostaining with anti-p53 antibody shows reactivity. (Magnification ×200).

Fig. 2. Histopathological and immunohistochemical analysis of CIN2.
a : HE staining. b : Immunostaining with anti-cathepsin D antibody shows reactivity. c : Immunostaining with anti-Ki-67 antibody has a labeling index of 56.57%. d : Immunostaining with anti-p53 antibody shows reactivity. (Magnification ×200).

Fig. 3. Histopathological and immunohistochemical analysis of CIN3.
a : HE staining. b : Immunostaining with anti-cathepsin D antibody shows reactivity. c : Immunostaining with anti-Ki-67 antibody has a labeling index of 86.36%. d : Immunostaining with anti-p53 antibody shows reactivity. (Magnification ×200).
Cathepsin D expression in uterine CIN

Cathepsin D staining had a fine granular appearance and was localized to the cytoplasm of dysplastic cells in CIN; cathepsin D expression was not detected in normal cervical squamous epithelium adjacent to the CIN. Three of the 17 CIN1 specimens (Fig. 1b), 16 of the 20 CIN2 specimens (Fig. 2b), and all 15 CIN3 specimens (Fig. 3b) were cathepsin D-positive (Table 1). There were significantly more cathepsin D-positive CIN2 and CIN3 specimens than CIN1 specimens (P < 0.01), but the incidence was not significantly different between CIN2 and CIN3 (Table 1).

Ki-67 in uterine CIN

Ki-67 staining was localized to the nuclei of dysplastic cells in CIN1, CIN2, and CIN3. A weak Ki-67 expression was detected in normal squamous epithelium adjacent to CIN, but positive cells accounted for less than 10% of the overall cell population. The Ki-67 labeling index was 26.41 ± 12.16% for the 17 CIN1 specimens (Fig. 1c), 50.35 ± 18.12% for the 20 CIN2 specimens (Fig. 2c), and 76.33 ± 18.39% for the 15 CIN3 specimens (Fig. 3c) (Table 1). It was significantly higher in CIN2 and CIN3 than in CIN1 (P < 0.01), and in CIN3 than in CIN2 (P < 0.01).

p53 in uterine CIN

p53 staining was localized to the nuclei of dysplastic cells in CIN1, CIN2, and CIN3. A weak p53 expression was detected in normal squamous epithelium adjacent to CINs, but positive cells accounted for less than 10%. Four of the 17 CIN1 specimens (Fig. 1d), 12 of the 20 CIN2 specimens (Fig. 2d) and 11 of the 15 CIN3 specimens (Fig. 3d) were p53-positive (Table 1). p53 expression was significantly higher in CIN2 and CIN3 than in CIN1 (P < 0.01), but there was no statistical difference between CIN2 and CIN3 (Table 1).

Correlation between cathepsin D and nuclear Ki-67 accumulation in dysplastic cells

In CIN1, the Ki-67 labeling index of cathepsin D-positive specimens (21.33 ± 6.60%) was not significantly different from that of cathepsin D-negative specimens (275 ± 12.78%) (Table...
Table 2. Relationship between cathepsin D expression and nuclear Ki-67 and p53 expression in dysplastic cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Histological grade</th>
<th>CD (+)</th>
<th>CD (-)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Ki-67 LI (%)</td>
<td>CIN1</td>
<td>21.33 ± 6.60</td>
<td>275 ± 12.78</td>
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<tr>
<td></td>
<td>CIN2</td>
<td>49.81 ± 19.79</td>
<td>52.5 ± 8.29</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>CIN3</td>
<td>76.33 ± 18.55</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>p53 (positive : negative)</td>
<td>CIN1</td>
<td>0 : 3</td>
<td>4 : 10</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>CIN2</td>
<td>12 : 4</td>
<td>0 : 4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>CIN3</td>
<td>11 : 4</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

CD: cathepsin D  
Ki-67 LI: Ki-67 labeling index

2). In CIN2, the Ki-67 labeling index of cathepsin D-positive specimens (49.81 ± 19.79%) was not significantly different from that of cathepsin D-negative specimens (52.5 ± 8.29%) (Table 2). All CIN3 specimens were cathepsin D-positive (Table 2).

Correlation between cathepsin D and nuclear p53 accumulation in dysplastic cells
In CIN1, none of the 3 cathepsin D-positive specimens was p53-positive, whereas 4 of the 14 cathepsin D-negative specimens were p53-positive (Table 2). This difference was not statistically significant. In CIN2, 12 of the 16 cathepsin D-positive specimens were p53-positive, whereas none of the 4 cathepsin D-negative specimens was p53-positive (Table 2). This difference was statistically significant (P < 0.01). All CIN3 specimens were cathepsin D-positive (Table 2).

Discussion
Cathepsin D expression is often detected in tumors showing invasive growth and at the invasive front of the tumor or around the cancer nests. While only a few carcinoma in situ show strong cathepsin D expression, this is a property of most tumors that invade the lamina propria 12). However, whether intraepithelial dysplastic cells express cathepsin D, suggesting an invasive potential, is still unclear.

A potential for invasive growth has not been reported for uterine cervical intraepithelial dysplastic cells. Our results indicate that the ratio of cathepsin D-positive cells increases significantly when CIN upgrades histologically from CIN1 to CIN2, and that such a ratio does not differ significantly between CIN2 and CIN3. Assessment of cathepsin D expression indicated that in most cases the dysplastic cells acquire an invasive growth potential when the grade of dysplasia progresses from CIN1 to CIN2. Furthermore, the proliferating potential of the dysplastic cells, assessed by accumulation of Ki-67 and p53, also increased when the degree of dysplasia progressed from CIN1 to CIN2, as reported by other laboratories 13-16).

The expression of cathepsin D correlates with the expression of Ki-67 in many invasive carcinomas 17-19). Cathepsin D promotes tumor cell proliferation by acting as an autocrine mitogen 17), and both cathepsin D expression and Ki-67 expression are important in the progression of malignancy 20). However, whether cathepsin D expression correlates with Ki-67 expression in intraepithelial dysplastic cells has not been established. In the current study, we could not demonstrate a relationship between cathepsin D expression and Ki-67 expres-
Cathepsin D expression in uterine CIN

Recent reports have revealed a significant positive correlation between cathepsin D expression and nuclear p53 expression in various tumors, indicating that tumor cells with nuclear p53 expression release signals that result in increased cathepsin D to degrade the extracellular matrix. However, in intraepithelial dysplastic cells, the relationship between cathepsin D expression and nuclear p53 expression is still unclear. In colorectal adenomas, we could demonstrate a correlation, and in the present study, we found that uterine CIN2 dysplastic cells expressing cathepsin D also expressed p53. Accordingly, our hypothesis is that p53 upregulates the expression of cathepsin D by dysplastic cells in CIN2.

**Conclusion**

Dysplastic cells have a higher expression of cathepsin D, Ki-67, and p53 in CIN2 than in CIN1, and this may suggest that the increase of potential malignancy occurs when CIN upgrades histologically from CIN1 to CIN2.

**References**


[Received November 21, 2005: Accepted December 14, 2005]