Immunohistochemical Evaluation of Cathepsin D Expression in Adenomas Containing Foci of Early Carcinoma: Correlation with Other Malignant Features

Hiroaki KURAI, Yuka KURAI, Keiko HENMI, Yuki CHO, Yenidunya SİBEL and Toshiaki KUNIMURA

Abstract: We evaluated the expression of cathepsin D (CD) in a series of colorectal adenomas with severe dysplasia containing foci of early carcinoma and compared the results to several histopathological and immunohistochemical features. Adenomas were obtained by endoscopic polypectomy from 33 patients. Twenty-four of the 33 adenomas contained well differentiated adenocarcinomas and 9 adenomas contained moderately-differentiated adenocarcinomas. High CD levels were observed in 25.0% of well differentiated adenocarcinomas and in 66.7% of moderately differentiated adenocarcinomas (P<0.05). Of the 12 adenocarcinomas with high CD expression levels, 4 had high CD expression in their adenomatous component (P<0.01), 6 showed positive staining for Ki-67 in their adenomatous component (NS) and 10 had positive staining for p53 in their adenomatous component (P<0.05). No significant association was seen between the level of CD expression and adenoma size. The expression of cathepsin D correlated significantly with differentiation, and with the levels of CD and p53 expression in the adenomatous component of the polyp.

Key words: colorectal tumor, adenocarcinoma, adenoma, cathepsin D, immunohistochemistry

Introduction

Tumors are known to secrete various tissue-specific proteolytic enzymes, with higher levels produced by malignant tumors than benign tumors or normal tissue. These enzymes play a role in the invasion by tumor cells through tissue barriers and into the blood and lymphatic systems. Cathepsin D (CD) is an aspartyl lysosomal protease, which is expressed in all cells throughout the body. Evidence for the role of CD in tumor progression has come from both in vivo and in vitro studies. In vitro, CD has the ability to digest extracellular matrix, including basement membrane components. In vivo, the prognostic value of CD expression has been studied in a variety of tumors. Endometrial carcinomas show a correlation between CD levels and the degree of differentiation as well as the depth of myometrial invasion. In gastric adenocarcinomas, a strong correlation between CD expression and the grade of differentiation has been reported. In colorectal...
tumors, there have been a few reports concerning the immunohistochemical expression of CD\(^5\)\(^-\)^\(^7\), however its role in early adenocarcinomas remains unclear. In this study, we evaluated the expression of CD in a series of colorectal adenomas with foci of early carcinoma, comparing their histopathological and immunohistochemical features.

**Materials and Methods**

Adenomas were obtained by endoscopic polypectomy from 33 patients. The patient age range was 40 to 85 years, and the male to female ratio was 23:10. Paraffin sections (3 mm) from the 33 polyps were stained with hematoxylin and eosin for histological review (Fig. 1) and classified according to the criteria of the Japanese Research for Cancer of the Colon and Rectum\(^8\)). Sixteen cases measured greater than 10 mm in the largest dimension, and 17 cases measured less than 10 mm. All adenomas showed severe dysplastic tubular adenoma with 24 containing well differentiated adenocarcinomas (Fig. 2a) and 9 containing moderately differentiated carcinomas (Fig. 2b). All adenocarcinoma cells were confined to the lamina propria and did not extend into the submucosa.

**Immunohistochemistry**

Consecutive 3 mm sections were dewaxed using xylene and rehydrated through a graded alcohol series. After reducing the endogenous peroxidase activity with absolute methanol containing 3 % \(\text{H}_2\text{O}_2\), sections were stained using the labeled streptavidin-biotin peroxidase complex technique (K0675, DAKO, Kyoto, Japan). Primary antibodies against cathepsin D (CD, H908, NICHIREI, Tokyo, Japan), Ki-67 (A0047, DAKO, Kyoto, Japan), and p53 (DO7, Novocastra, Newcastle, UK), were applied for 18 hours at 4 °C. The antibody binding was visualized using benzidine and the sections were lightly counterstained with hematoxylin for light microscopy.

**Immunohistochemical evaluation**

The immunostaining was quantified as the percentage of positive tumor cells in relation to the total number of cells in representative fields of both adenomas and adenocarcinomas. In most cases the adjacent normal mucosa was also examined. We separated the cases into two groups, denoted as “high” and “low”, according to the percentage of positive tumor

![Fig. 1. Low power view of a foci of adenocarcinoma in an adenoma Arrow indicates adenocarcinoma. (×2.5, hematoxylin and eosin staining)](image)
cells among more than five hundred tumor cells. The arbitrary cut-off line was 10% for
CD expression, 50% for Ki-67 expression, and 5% for p53 expression.

Statistical analysis
The association of continuous variables was confirmed using a non-parametric test for two
independent samples. P-values < 0.05 were considered statistically significant.

Results

Immunohistochemistry
CD immunopositive staining was found in the cytoplasm of adenocarcinoma cells in all
cases of our colorectal adenoma series. High CD expression levels were observed in 4/33
(12.1%) of adenomas and in 12/33 (36.4%) of adenocarcinomas (P < 0.05) (Fig. 3a)
(Table 1). Low CD expression levels were detected in 29/33 (87.9%) of adenomas and in
21/33 (63.6%) of adenocarcinomas. CD was not expressed in normal colorectal epithelial
cells. High nuclear Ki-67 expression, indicating proliferative activity, was observed in 14/33
(42.4%) of adenomas and in 33/33 (100%) of adenocarcinomas (p < 0.01) (Fig. 3b). Weak
Ki-67 expression was detected in normal colorectal epithelial cells. Overexpression of
p53 protein, indicating p53 inactivation, was observed in 18/33 (54.5%) of adenomas and
in 33/33 (100%) of adenocarcinomas (p < 0.01) (Fig. 3c). No p53 expression was
observed in normal colorectal epithelial cells.
Specific correlation between CD expression and histopathology: There was no significant correlation between the level of CD expression and adenoma size. Of the adenomas with high CD expression levels in the adenocarcinoma component, 4/16 (25%) measured >10 mm in diameter, and 8/17 (47.1%) measured <10 mm. However, a significant correlation was observed between CD expression levels and tumor differentiation. Six of 24 (25.0%) well differentiated adenocarcinomas and 6/9 (66.7%) moderately differentiated cases showed high levels of CD protein expression (p<0.05) (Table 1).

Specific correlation between CD expression, and Ki67 and p53 immunostaining patterns:
Table 1. Relationship between cathepsin D expression and histopathological and immunohistochemical features

<table>
<thead>
<tr>
<th></th>
<th>CD in adenocarcinoma</th>
<th>CD in adenocarcinoma</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td></td>
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<tr>
<td>Tumor dimension</td>
<td></td>
<td></td>
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<tr>
<td>&gt;10 mm : &lt;10 mm</td>
<td>4 : 8</td>
<td>12 : 9</td>
<td>NS</td>
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<td>Differentiation in adenocarcinoma</td>
<td></td>
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<tr>
<td>well : moderate</td>
<td>6 : 6</td>
<td>18 : 3</td>
<td>&lt;0.05</td>
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<td>Ki-67 in adenocarcinoma</td>
<td></td>
<td></td>
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<tr>
<td>High : Low</td>
<td>12 : 0</td>
<td>21 : 0</td>
<td>NS</td>
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<td>p53 in adenocarcinoma</td>
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<td>High : Low</td>
<td>12 : 0</td>
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<td>NS</td>
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<tr>
<td>CD in adenoma</td>
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<tr>
<td>High : Low</td>
<td>4 : 8</td>
<td>0 : 21</td>
<td>&lt;0.01</td>
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<td>Ki-67 in adenoma</td>
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<td>High : Low</td>
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<td>p53 in adenoma</td>
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<td>High : Low</td>
<td>10 : 2</td>
<td>8 : 13</td>
<td>&lt;0.05</td>
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CD : cathepsin D expression. “High” when more than 10% of the tumor cells are positive, “Low” when less than 10% are positive.

Ki-67 : Ki-67 expression. “High” when more than 50% of the tumor cells are positive, “Low” when less than 50% are positive.

All adenocarcinomas in this series expressed either high or low levels of CD, showed high nuclear staining for Ki-67 and stained positive for p53. Of the 12 adenocarcinomas with high CD expression levels, 4 had high CD expression in their adenomatous component (P<0.01), 6 showed positive staining for Ki-67 in their adenomatous component (NS) and 10 had positive staining for p53 in their adenomatous component (P<0.05) (Table 1).

Discussion

The adenoma-carcinoma sequence is widely accepted as the evolutionary paradigm for colorectal carcinoma, and it is generally agreed that most colorectal carcinomas arise from pre-existing adenomas rather than by de novo carcinogenesis\(^\text{10,11}\). Ki-67 and p53 immunohistochemical studies have revealed differences in the malignant potential of adenomas and adenocarcinomas. In agreement with other studies, we found significantly higher Ki-67 expression levels and p53 nuclear staining, indicating higher proliferative activity and p53 inactivation, respectively, in adenocarcinomas than in adenomas\(^\text{11-18}\).

Recent immunohistochemical studies using CD expression levels to predict the malignant potential of tumor cells found no significant correlation between CD expression in advanced colorectal carcinomas and tumor progression, invasion or metastasis\(^\text{19-22}\). The invasive growth potential of the early colorectal tumor cells, indicated by CD expression\(^\text{2}\), therefore remains unclear. We investigated the expression of CD in intramucosal adenocarcinomas arising in adenomas. We found no correlation between CD expression levels and adenoma size but found a significant correlation between expression of CD and the extent of
adenocarcinoma differentiation. Moreover, we found a significant correlation in the levels of CD expression between adenocarcinoma cells and adenoma cells. CD expression as a predictor of malignant potential may be useful in distinguishing adenocarcinomas from adenomas in intramucosal lesions.

The expression of CD protein is reported to correlate with both Ki-67 and p53 expression. CD promotes tumor cell proliferation by acting as an autocrine mitogen, and both CD expression and p53 expression are important in the progression from adenoma to invasive carcinoma suggesting that adenocarcinoma cells expressing CD are more aggressive than tumor cells not expressing CD. In the current study, we failed to demonstrate a relationship between CD expression and proliferating potential or p53 expression in adenocarcinomas, because all cases had high expression levels of Ki-67 and p53. However, a significant correlation was found between the expression of CD and p53 in the adenomatous components of the polyps. To the best of our knowledge, this is the first report describing this relationship.

In conclusion, our results show that CD is expressed in a high proportion of colorectal tumors. Further estimation of cathepsin D, especially in combination with other markers, may provide clues to the biological behavior of colorectal cancer.

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References


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