Abstract

The majority of the adenocarcinomas arising in Barrett esophagus manifest clinically at an advanced stage and have a poor prognosis. As a result of this poor prognosis, much attention has been directed toward the exploration of markers for neoplastic progression in Barrett esophagus. The objective of the present study was to determine the expression of beta-catenin by immunohistochemical analysis in 70 adenocarcinomas developed in Barrett esophagus and to examine its relationship to various prognostic factors currently in use. Abnormal beta-catenin expression, consisting of the loss of membranous staining and the appearance of the nuclear staining, was found in 43 cases (61%). Of patients with the 43 tumors showing abnormal beta-catenin expression, 25 (58%) survived more than 1 year. In contrast, only 7 (26%) of 27 patients with tumors showing normal beta-catenin expression survived longer than 1 year. Most of the superficial (Tis-T1) tumors (83% [10/12]) exhibited abnormal beta-catenin expression compared with only 53% (31/58) in the T2-T3 group. These results suggest a possible correlation among beta-catenin expression, tumor stage, and length of survival as prognostic factors in patients with adenocarcinoma in Barrett esophagus.

Barrett esophagus is a condition in which the normal stratified squamous epithelium in the distal esophagus is replaced by metaplastic columnar epithelium. This condition predisposes patients to the development of esophageal adenocarcinoma at a rate of 30- to 125-fold higher than in the general population. Unfortunately, most malignant neoplasms resulting from Barrett esophagus are detected at an advanced stage in which they are incurable, with an overall 5-year survival rate of less than 10%.

Like other malignant neoplasms, those in the esophagus are thought to result from the accumulation of certain genetic alterations that affect normal control of cell growth and differentiation. Many clinicopathologic studies have analyzed the dysplasia-carcinoma sequence to find a marker that would predict further evolution of Barrett esophagus to allow intervention before the disease becomes incurable. To date, few authors have studied the relationship between genetic alterations and prognosis of esophageal adenocarcinoma.

beta-Catenin is an oncoprotein that mediates cell-cell adhesion via the transmembrane E-cadherin–catenin complex and contributes to carcinogenesis when the APC/beta-catenin/Tcf signal transduction pathway is disrupted. In adenocarcinoma of prostate, breast, lung, stomach, and colon and squamous cell carcinoma of the esophagus and head and neck, decreased E-cadherin and/or alpha-catenin expression was a useful prognostic factor. Similarly, decreased beta-catenin expression was observed and reported in several gastrointestinal cancers. In most of these studies, reduced beta-catenin expression was correlated with a poor survival rate except in bladder carcinoma in which it is associated with a better prognosis. The majority of studies have
focused on the reduction of membranous staining until recently, when focal nuclear beta-catenin staining was considered and described in high-grade dysplastic Barrett esophagus and adenocarcinoma.14,15

The aims of the present study were to investigate the pattern of expression of beta-catenin by immunohistochemical analysis in adenocarcinomas in Barrett esophagus (membranous and nuclear staining) and to determine potential correlation with prognostic factors such as tumor differentiation, depth of invasion, lymph node metastases, and survival.

Materials and Methods

From our files, we selected 70 cases (10 women and 60 men) of esophagectomy specimens obtained between 1970 and 1996 in which adenocarcinoma developed in Barrett esophagus. The average age at esophagectomy was 63.6 years (range, 41-85 years). No patients had received radiation or chemotherapy before or after surgery. All gross and histologic specimens were reexamined by 2 pathologists (M.C.O. and C.F.). Clinical data were obtained from pathology reports, referring clinicians, or the Tumor Registry, Lausanne, Switzerland.

H&E-stained sections were screened to identify the lesions and their locations. Barrett esophagus was defined by the presence of readily identifiable goblet cells above the gastroesophageal junction (intestinal metaplasia). Invasive adenocarcinoma was diagnosed when atypical cells or glands infiltrated into the submucosa. The adenocarcinomas were graded as well-differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3), and the depth of invasion was determined according to the International Union Against Cancer TNM classification of malignant tumors.16

From each sample, 4-µm paraffin sections were cut and mounted on coated slides. Immunohistochemical analysis was performed using an avidin-biotin-peroxidase complex method as described previously.17 A colorectal carcinoma with a high membranous (M+) and no nuclear (N–) expression of beta-catenin was used as positive control, while negative control slides were obtained by using phosphate-buffered saline instead of primary antibody. Results were analyzed by 2 independent observers (M.C.O. and C.F.) and discussed in discrepant cases. Membranous and nuclear expressions of beta-catenin were assessed, using the staining intensity of normal mucosa present on the same slide as an internal standard. Cytoplasmic immunoreactivity was rather variable and not clearly related to the shift from membranous to nuclear staining; therefore, it was not considered in the present study.

For further evaluation, the results were placed into 2 groups: normal and abnormal staining patterns. Expression of beta-catenin was considered normal when the majority of the cells displayed membranous staining without nuclear staining (N–M+). Abnormal expression was seen in adenocarcinomas and comprised 4 immunostaining patterns: tumors with nuclear staining in more than 5% of the cells as previously described18 and with membranous staining less intensive than (M–), equal to (+), or more intensive than (+++) the membranous staining of the normal adjacent epithelium (N+M–, N+M+, N+M++) and tumors with neither membranous nor nuclear staining (N–M–).

Possible correlations between the pattern of expression of beta-catenin and survival, depth of invasion, grade of tumor differentiation, and lymph node metastases were analyzed statistically by means of the Fisher exact test. A P value of less than .05 was considered statistically significant.

Results

All 70 tumors developed from Barrett esophagus. Of the adenocarcinomas, 18 were well-differentiated (grade 1), 18 were moderately differentiated (grade 2), and 34 were poorly differentiated (grade 3). Two were considered carcinoma in situ (Tis), 10 invaded the lamina propria or the submucosa (T1), 6 infiltrated the muscularis propria (T2), and 52 reached the adventitia (T3). There were no cases of tumor invasion into the adjacent structures (T4). All margins of resection were negative, and no distant metastases were found at diagnosis. Only 32 (46%) patients with adenocarcinoma survived for more than 1 year. The 1-year survival rate was 75% for Tis and T1 tumors and decreased to 67% for T2 tumors and 37% for T3 carcinomas. One-year survival was 56% for well- and moderately differentiated tumors and only 35% for poorly differentiated tumors. The most important prognostic factor was the presence of lymph node metastases, with a 1-year survival of 76% for the group without lymph node involvement and only 30% for patients with lymph node metastases Table II. Nuclear staining with normal (N+M+), negative (N–M–), or increased (N+M++) membranous beta-catenin expression was present in 39 adenocarcinomas. Four adenocarcinomas showed no staining, neither membranous nor nuclear Image 1. Since N–M+ (Image 1) represents the normal epithelial expression of beta-catenin, all 43 tumors (61%) clearly showed abnormal beta-catenin staining. In 27 patients (38%), tumors showed a normal pattern of beta-catenin expression (no nuclear staining but membranous staining [N–M+]).

Correlation with established prognostic factors is given in Table II. Of the 43 patients with tumors showing abnormal expression of beta-catenin, 25 (58%) survived...
more than 1 year compared with only 7 (26%) of 27 patients with normal beta-catenin expression \( (P = .008) \). The patient with the longest survival time (10 years) was in the tumor group with abnormal beta-catenin expression, although this tumor had infiltrated the muscularis propria (T3) and lymph node metastasis had developed. This tendency toward better prognosis for the tumors with abnormal beta-catenin expression also was observed for the depth of infiltration. In fact, most of the superficial tumors showed abnormal beta-catenin expression (Tis-T1, 10/12 cases [83%]; T2-T3, 31/58 cases [53%]), which is at the limit of significance \( (P = .05) \). However, we did not find a significant correlation between beta-catenin expression and tumor grade \( (P = .06) \) or lymph node metastases \( (P = .29) \).

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>No. of Cases</th>
<th>Survival &gt;1 y *</th>
</tr>
</thead>
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<tr>
<td>Grade</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>18</td>
<td>10 ( (56) )</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>10 ( (56) )</td>
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<tr>
<td>3</td>
<td>34</td>
<td>12 ( (35) )</td>
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<td>Lymph node metastasis</td>
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<td></td>
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<tr>
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<td>43</td>
<td>13 ( (30) )</td>
</tr>
<tr>
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<td>25</td>
<td>19 ( (76) )</td>
</tr>
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<td>Infiltration depth</td>
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<td></td>
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<tr>
<td>Tis-T1</td>
<td>12</td>
<td>9 ( (75) )</td>
</tr>
<tr>
<td>T2</td>
<td>6</td>
<td>4 ( (67) )</td>
</tr>
<tr>
<td>T3</td>
<td>52</td>
<td>19 ( (37) )</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

Degradation of beta-catenin is prevented by mutation of APC or beta-catenin or by mutation or down-regulation of E-cadherin. Then beta-catenin moves to the nucleus and coactivates transcription factor Tcf, in addition to other cellular elements, to mediate transcription of transcription factor myc. Myc induces transcription of telomerase, which provides immortality to cells and also induces a yet-unidentified factor that releases p27 and thus inhibits cyclin cdk2 activity leading to growth inhibition. The presence of beta-catenin nuclear staining also has been observed in colorectal adenocarcinoma, generally related to APC mutations.4,30 These findings suggest that intracellular distribution of beta-catenin may have a role in the complex signal transduction pathways involved in cell growth and tumor formation. Details of the mechanisms of abnormal expression of the E-cadherin–catenin complex remain unclear. Even if several reports are contradictory, abnormal expression of E-cadherin and beta-catenin often is reported, suggesting a correlation with prognosis that warrants further exploration. Many contradictions in the literature may have been produced by difficulties in interpretation of beta-catenin staining. Most authors have considered reduced membranous staining alone, while others have focused on nuclear staining and included the examination of cytoplasmic immunoreactivity, which was found to be rather variable.

A unique feature of the present study is that we considered any nuclear staining aberrant, regardless of membranous staining (N+M–, N+M+, N+M++). We also included in this group the cases without any staining (N–M–). Our results confirm the abnormal expression of beta-catenin found in most esophageal adenocarcinomas (43/70 [61%]) from Barrett mucosa consistent with previous descriptions.15 The presence of abnormal expression of beta-catenin was significantly correlated with a better survival ($P = .008$). The correlation of beta-catenin expression was not associated significantly with tumor differentiation or lymph node metastases. The correlation with depth of infiltration was at the limit of significance ($P = .05$).

The difference between our results and the conclusions of previous studies may be due to different interpretations of immunohistochemical results. Indeed, we considered the most important and unequivocal changes of beta-catenin staining in neoplastic cells. Absence of APC and beta-catenin mutations17,33 has been described in some tumors with abnormal expression of beta-catenin. Furthermore, this abnormal expression is not correlated with lymph node metastasis, which is the most important prognostic factor in esophageal adenocarcinoma. This phenomenon suggests that beta-catenin has a role in the regulation of adhesion cells and also may interact with tumor growth. This hypothesis corroborates the suggestions of many studies that suggest the presence

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation Between beta-Catenin Expression and Accepted Prognostic Factors</th>
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<tbody>
<tr>
<td>beta-Catenin</td>
<td>Normal Expression</td>
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<tr>
<td>1-2</td>
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<td>Infiltration depth</td>
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<tr>
<td>T2-T3</td>
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<tr>
<td>Survival</td>
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<td>&gt;1 y</td>
<td>7</td>
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<tr>
<td>≤1 y</td>
<td>20</td>
</tr>
</tbody>
</table>

None of the 30 adenocarcinomas analyzed in a previous study showed a beta-catenin mutation.19

Discussion

Several authors have studied genetic features associated with esophageal cancer in search of parameters that more accurately assess the risk of progression of Barrett esophagus to adenocarcinoma.20-28 Loss of epithelial integrity due to abnormal expression of E-cadherin, alpha-catenin, or beta-catenin has been shown in a number of tumors and cell lines.18,29-32 It has been hypothesized that the loss of normal expression or function of any component of this complex may contribute to the malignant phenotype. The association of the cytoplasmic domain of cadherin with the actin cytoskeleton via catenin is essential for cell-cell adhesion. In carcinomas, the function of the E-cadherin–catenin complex in the maintenance of epithelial integrity may be lost, resulting in increased invasiveness and metastatic ability of malignant cells. In esophageal adenocarcinoma, Krishnadath et al6 showed that reduced beta-catenin expression is correlated with poor differentiation (high grade) and shorter survival but not disease stage. Furthermore, they also showed that all 3 proteins of the E-cadherin–catenin complex are expressed in lymph node metastases, indicating that expression of the complex is reestablished to develop tissue architecture. The findings of this study6 and several others16,32,33 indicate that the shift from membranous to nuclear staining is not caused by mutations in the beta-catenin gene. In normal cells, intercellular binding mediated by E-cadherin molecules results in binding of beta-catenin to E-cadherin on the cellular membrane or to destruction of beta-catenin by APC-GSK3 complex formation.34 In both cases, entry of beta-catenin into the nucleus is prevented.

APC and beta-catenin mutations have been described in some tumors with abnormal expression of beta-catenin. Furthermore, this abnormal expression is not correlated with lymph node metastasis, which is the most important prognostic factor in esophageal adenocarcinoma. This phenomenon suggests that beta-catenin has a role in the regulation of adhesion cells and also may interact with tumor growth. This hypothesis corroborates the suggestions of many studies that suggest the presence
of a dual role for the E-cadherin–catenin complex as a tumor suppressor and an oncogene in human cancers.33,35-38

Even if our study is based only on a moderate number of cases, the results suggest that abnormal beta-catenin expression could be used as a marker for prognosis in advanced adenocarcinomas. Generally the survival rate of Barrett adenocarcinoma is very poor and apparently is worsened when tumors show normal beta-catenin expression. Normally we would expect that loss of membranous staining would be associated with higher invasiveness of tumors if the theory of disruption of cell-cell adhesion were applied. The only explanation for these results is the interaction with tumoral growth by way of apoptosis, which then would have a substantial impact on containment of the tumor.37,38

The present study shows that abnormal expression of beta-catenin is a common event in adenocarcinomas developed in Barrett esophagus and correlates with better survival. These results confirm the assessment that reduced membranous expression and enhanced nuclear expression of beta-catenin frequently are observed in human malignant neoplasms and emphasize that clinical implications of aberrant beta-catenin expression are more complex than expected.

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References


