CDX2 and Villin Are Useful Markers of Intestinal Metaplasia in the Diagnosis of Barrett Esophagus

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Abstract

The identification of intestinal metaplasia (IM) in the esophagus is necessary for the selection of patients with Barrett esophagus (BE) for surveillance. We studied 108 esophageal biopsy and resection specimens, clinically diagnosed as BE, and stained them for CDX2, villin, HepPar-1, and cytokeratin (CK) 7 to investigate sensitivity for identifying IM. H&E-stained sections showed definite goblet cells in 94 cases. CDX2 and villin were positive in all 94 cases. Of 38 cardia- and 9 fundic-type mucosa samples associated with BE, 13 (34%) and 0 (0%) displayed CDX2 positivity and 21 (55%) and 1 (11%) displayed villin positivity, respectively. HepPar-1 was positive in 54 (57%) of 94 cases with IM and negative in the associated cardia- and fundic-type mucosa. A full-thickness CK7 staining pattern was present in 90 (96%) of samples with IM and 22 (58%) and 0 (0%) of the associated cardia- and fundic-type mucosa, respectively. None of 20 control samples of morphologically normal gastric mucosa stained for CDX2 or villin. CDX2 and villin are sensitive markers for early-stage IM and can supplement the histologic identification of this premalignant condition in the esophagus.

Barrett esophagus (BE) is an established precursor of esophageal adenocarcinoma, a tumor with a rapidly increasing incidence in most Western countries.1 BE was found in 1.6% of the general population and in 10% of patients who underwent endoscopy for symptoms of gastroesophageal reflux disease (GERD).2 The risk of esophageal adenocarcinoma in patients with BE seems to be approximately 30- to 125-fold greater than that in the general population, with an estimated incidence of 1 in 52 to 1 in 441 patient-years.3-6

Because of the poor prognosis of esophageal adenocarcinoma, management of this disease through surveillance of patients with BE and identification of patients with a high risk of developing adenocarcinoma is the conventional approach. However, the definition of BE remains controversial. The current definition by the World Health Organization states that “BE is restricted to cases with histologically confirmed intestinal metaplasia…” with further description of BE as being “characterized by two different types of cells, ie, goblet and columnar cells…”7 The identification of goblet cells, thus, remains the “gold standard” for the diagnosis of intestinal metaplasia (IM) and BE.

Goblet cells are not the only cells present in metaplastic epithelium, which may be flat or villiform, with other cell types including mucous and absorptive cells and Paneth cells. Some authors believe that the presence of mucous glands in the gastroesophageal junction area represents an acquired form of metaplastic epithelium that develops as a result of GERD.8,9 There is also evidence to suggest that the recently described multilayered epithelium comprising both squamous and columnar epithelium represents a transitional stage in the development of BE,10 further adding to the speculation that the presence of goblet cells may not be the earliest indicator of...
IM in the esophagus. Cases with columnar-lined mucosa but without demonstrable goblet cells have an increased risk of harboring undetected IM or developing IM with time.11

In 2005, the British Society of Gastroenterology (BSG) guidelines for the diagnosis and management of BE stated, “The presence of areas of intestinal metaplasia (IM), although often present, is not a requirement for diagnosis.”12 The BSG guidelines further stated that “The insistence on identification of intestinal metaplasia to establish a diagnosis of ‘Barrett’s oesophagus’ or to signify malignant potential is not supported by UK pathological opinion which believes that intestinal metaplasia can always be identified in endoscopically-visible columnar metaplasia providing a sufficient number of biopsies are taken over an adequate time-scale.”13

The increasing incidence of esophageal adenocarcinoma raises the possibility that IM in the esophagus is underdiagnosed. It is, therefore, pertinent to search for other markers of IM besides goblet cells, the identification of the latter being recognized to be influenced by sampling errors. To this end, we examined the specificity and sensitivity of cytokeratin (CK) 7, HepPar-1, CDX2, and villin as markers of IM in the esophagus.

Materials and Methods

We obtained 108 esophageal specimens from biopsies and resections, performed from 1999 to 2006 and diagnosed purely on clinical grounds as BE, from the database of the Division of Anatomical Pathology, Hunter Area Pathology Service, Newcastle, Australia. Clinical data and pathologic slides were reviewed in all cases. The age of the patients ranged from 21 to 93 years (mean ± SD, 63.0 ± 14.6 years) with a male/female ratio of 3.1:1. We selected 20 gastric biopsy specimens that contained normal-appearing fundic mucosa with no microscopic evidence of inflammation or IM, obtained from patients for clinical reasons unassociated with GERD, from the files as control samples for the immunostains.

IM was defined as the presence of goblet cells in columnar mucosa. Cardia-type mucosa was defined as columnar mucosa with the absence of goblet cells as the only histologic difference compared with IM. Fundic-type mucosa was defined as columnar mucosa with oxyntic glands and absence of goblet cells.

Antigen retrieval conditions and visualization systems varied with the antibody used. Briefly, for CDX2 (clone AMT28, dilution 1:100; Novocastra Laboratories, Newcastle upon Tyne, England), the slides were treated in 0.01 mol/L EDTA, pH 8.0, in a microwave oven at 98°C for 20 minutes; for CK7 (clone OV-TL12/30, dilution 1:1,500; BioGenex, San Ramon, CA), the slides were treated in 0.01 mol/L citric acid, pH 6.0, in a microwave oven at 98°C for 20 minutes; for anti–hepatocyte-specific antigen (HepPar-1, clone OCH1E5, dilution 1:50; Novocastra), the sections were incubated in protease 2 (Ventana Medical Systems, Victoria, Australia) at 37°C for 2 minutes followed by microwave antigen retrieval in 0.01 mol/L citric acid buffer, pH 6.0, at 98°C for 20 minutes; and for villin (clone CWWB1, dilution 1:300; Novocastra), the sections were treated in 0.01 mol/L citric acid, pH 6.0, in a microwave-heated high-pressure cooker at 110°C for 20 minutes. Staining was performed in a Bond-Max automatic immunostainer (Vision BioSystems, Victoria, Australia) or a Ventana ES automatic immunostainer (Ventana Medical Systems). Negative control samples included omission of the primary antibody and the use of 5% goat serum as the primary antibody. Known positive tissue sections served as positive control samples.

All immunohistochemical stains were separately evaluated by 2 pathologists (X.Y.S. and A.S.-Y.L.). The antigens were localized as follows: CDX2 was nuclear. Villin stained the brush border of the superficial columnar epithelium and luminal borders of the glandular epithelium with or without cytoplasmic staining. HepPar-1 staining was granular and cytoplasmic. CK7 staining occurred in 3 patterns: full-thickness staining of columnar mucosa, diffuse staining of superficial columnar epithelium, and patchy staining only.

Statistical analysis was performed using the SPSS version 11 statistical package (SPSS, Chicago, IL). The χ² or a 2-sided Fisher exact test was used for comparison of the positive rates of different markers, and P values of less than .05 were regarded as significant.

Results

Alcian blue–periodic acid–Schiff stains confirmed the presence of definite goblet cells in 94 of 108 cases. Accompanying cardia-type mucosa was identified in 38 and fundic-type mucosa in 9 of the 94 cases. These occurred as separate pieces of tissue included in the single esophageal sample. The remaining 14 cases that were clinically diagnosed as short-segment BE but failed to show goblet cells in the columnar-lined epithelium were also studied. Cardia-type mucosa was present in all and fundic-type mucosa was found in 7 of the 14 cases (Table II).

CDX2, Villin, and HepPar-1 Expression

In BE, CDX2 and villin staining highlighted goblet cells and were positive in 93 (100%) of 93 cases showing IM, ie, displaying goblet cells (except in 1 case in which there was insufficient tissue for staining) (Image II). In the accompanying cardia-type mucosa, which showed no morphologic differentiation of goblet cells, CDX2 and villin expression were focally present in 13 (34%) and 21 (55%) of 38 cases, respectively (Image III).
In the accompanying fundic-type mucosa, CDX2 expression was not seen, whereas focal villin expression was present in 1 case. Larger numbers of villin-positive cells were observed in the cardia- and fundic-type mucosa as compared with CDX2, but the difference was not significant ($P = .0548$). Granular cytoplasmic HepPar-1 expression was present only in columnar mucosa with goblet cells, but the staining of goblet cells and adjacent mucous cells was weak and variable. HepPar-1 was, therefore, of relatively poor sensitivity (53/92 [58%]; $P < .05$ vs CDX2 and villin).

In the biopsy specimens that did not contain goblet cells, the frequency of CDX2 and villin expression in cardia- and fundic-type mucosa was similar to that observed in BE ($P > .05$). There was no staining for CDX2, villin, or HepPar-1 in the 20 control samples.

### CK7 Staining

Most of the cases with IM had a full-thickness CK7 staining pattern (90/94 [96%]); however, the pattern of staining could be variable in the same biopsy specimen, whereas all fundic-type mucosa demonstrated superficial (3/9 [33%]) or patchy (6/9 [67%]) staining. Of 38 cardia-type mucosa samples, 22 (58%) showed a full-thickness staining pattern, 11 (29%) showed superficial staining, and 5 (13%) showed patchy staining. The staining patterns of CK7 in the 3 types of mucosa were significantly different ($P < .01$). CK7 staining patterns in cardia- and fundic-type mucosa among the non-BE cases were similar to their counterparts that accompanied BE ($P > .05$).

### Discussion

In past decades, there has been a remarkable change in the epidemiology of esophageal cancer. Previously rare, adenocarcinoma of the esophagus and gastroesophageal junction is now the most common esophageal cancer, and in the United States, the incidence is increasing faster than that of any other malignancy. As an established precursor to esophageal adenocarcinoma, BE attracts more attention than ever. By definition, BE is characterized by the replacement of the squamous epithelium of the esophagus with columnar epithelium that contains goblet cells (IM), the latter being the prerequisite for diagnosis.

Columnar-lined esophageal tissue alone in the absence of goblet cells currently fails to meet the criteria for BE, and such cases are excluded from endoscopic surveillance and not regarded as precursors to malignant change. However, an

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**Table 1**

<table>
<thead>
<tr>
<th>Mucosa Type</th>
<th>Barrett Esophagus (n = 94)</th>
<th>Columnar-Lined Esophagus Without Goblet Cells (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal metaplasia (goblet cells present)</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>Accompanying cardia type</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>Accompanying fundic type</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

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**Table 2**

**Image 1** Barrett esophagus stained for CDX2 and villin. There is nuclear staining of goblet cells and columnar cells for CDX2 (A, ×200), whereas villin is sharply localized to the brush border and cytoplasm of goblet and columnar cells (B, ×200).
**Image 2** Cardia-type mucosa associated with Barrett esophagus. A, The cardia-type mucosa has a lining of columnar cells, pits, and glands and is different from Barrett mucosa only by the absence of goblet cells (H&E, ×100). B, Alcian blue–periodic acid–Schiff (PAS) staining shows positivity for PAS but absence of alcian blue uptake (×100). C, Focal staining for CDX2 is present (arrow; ×200). D, There is strong labeling of the columnar cells for villin in the cytoplasm and brush borders of the columnar cells (×40), highlighted in the inset (×100).

**Table 2**
CDX2, Villin, and HepPar-1 Expression in Columnar Mucosa

<table>
<thead>
<tr>
<th>Staining</th>
<th>Intestinal Metaplasia</th>
<th>Cardia-Type Mucosa</th>
<th>Fundic-Type Mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>93/93 (100)</td>
<td>13/38 (34)†</td>
<td>0/9 (0)†</td>
</tr>
<tr>
<td>CDX2</td>
<td>93/93 (100)</td>
<td>21/38 (55)†</td>
<td>1/9 (11)†</td>
</tr>
<tr>
<td>Villin</td>
<td>54/94 (57)</td>
<td>0/38 (0)†</td>
<td>0/9 (0)†</td>
</tr>
<tr>
<td>HepPar-1</td>
<td>—</td>
<td>2/14 (14)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Columnar mucosa without goblet cells</td>
<td>6/14 (43)</td>
<td>1/7 (14)</td>
<td></td>
</tr>
<tr>
<td>CDX2</td>
<td>—</td>
<td>0/14 (0)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Villin</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepPar-1</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as number/total (percentage).
† Difference significant compared with intestinal metaplasia.
increased risk of harboring undetected intestinal metaplasia or developing intestinal metaplasia with time has been reported in such cases, and sampling errors are a recognized and common problem. A recent epidemiologic study revealed that irrespective of the increase in the proportion of esophageal biopsy specimens diagnosed with BE, the incidence of adenocarcinoma of the esophagus continued to increase disproportionately. Of 105,283 patients with a first-time esophageal biopsy, 33,365 had BE, 6,168 had squamous cell carcinoma, and 9,854 had adenocarcinoma of the esophagus. Although the number of esophageal biopsies increased by 21% among men and 6% among women between 1992 and 2003, with the proportion of biopsy specimens diagnosed with BE increasing by a larger extent, 33% for men and 25% for women, the incidence of adenocarcinoma of the esophagus increased disproportionately, by 28% and 22%, respectively.

Compared with the situation with cervical cancer for which incidence decreased dramatically after the introduction of screening programs in developed countries in the 1960s and 1970s, the increasing incidence of esophageal adenocarcinoma suggests that endoscopic surveillance is not sufficiently effective, and underdetection of cases predisposed to malignant change may be the likely explanation for this observation. This underdiagnosis of BE may result from too restrictive a definition and indicates the need to identify changes of IM without the presence of goblet cells. The development of goblet cells may not be the earliest indicator of intestinal metaplasia; for example, the recently described multilayered epithelium of mixed squamous and columnar cells has been implicated as a transitional stage in the development of BE. As such, phenotypic markers of intestinal epithelial differentiation may represent more sensitive means of identifying IM instead of reliance on the identification of goblet cells.

One study of IM in gastric mucosa indicated that the process starts from a heterogeneous cell population with gastric and intestinal phenotypes that gradually convert into a pure intestinal phenotype. The mixed gastric- and intestinal-type mucosa contains gastric- and intestinal-type mucin and villin. With the progression from mixed-type glands to intestinal-type glands, the expression of intestinal-type mucin and villin increases with a reciprocal decrease in the expression of gastric-type mucin. The development of IM in the background of columnar-lined esophageal tissue may mimic this process in the stomach. Differentiation into goblet cells may, therefore, represent end-stage metaplasia into intestinal-type mucosa, and the commitment to this neoplastic pathway occurs at an earlier stage that may be recognized with the help of specific biomarkers.

Villin is an actin-binding cytoskeletal protein in the microvillus core of the brush border. Its expression can be induced by long-term acid exposure and correlated with the development of differentiated polarized cells that contain a brush border and microvillus inclusions. The reported frequency of villin expression in BE varies from 73% (22/30) to 100% (21/21). Villin is an end-differentiated marker of intestinal cells, and the protein needs to be of a sufficient quantity to result in a mature brush border. We found villin expression to be present in all 93 cases that contained goblet cells. Besides goblet cells, villin was expressed in the brush border and cytoplasm of superficial columnar epithelium and luminal borders of glands containing goblet cells. An in vitro study demonstrated the rate of villin synthesis to be dramatically increased in the course of enterocyte differentiation, remaining remarkably stable after synthesis. Immunofluorescence and immunogold labeling showed villin localized to the brush border area of differentiated cells but remaining diffusely distributed in undifferentiated cells.

In our cases, both staining patterns were present not only in columnar-lined mucosa with goblet cells but also in cases without goblet cells (cardia-type mucosa). This observation suggests that cells expressing villin in the brush border may represent changes of IM even in the absence of goblet cell differentiation, whereas cytoplasmic expression alone may indicate an earlier stage of intestinal differentiation. None of the fundic cells in gastric biopsy specimens showed labeling with villin.

The homeobox protein CDX2 is a transcription factor involved in early intestinal differentiation and regulates the transcription of several intestinal genes, including MUC2. Theoretically, its expression in IM should occur earlier than changes in mucin type. Several studies have demonstrated CDX2 expression localized to the goblet cells of BE; however,
its expression has also been reported in nongoblet columnar epithelium in a frequency varying from 0% to 100%.22,28-31 The reason for these wide reported differences is difficult to explain, and variations in sampling and sensitivity of immunostaining may be contributory. Our study showed that in the presence of goblet cells, associated cardia-type mucosa in the same sample was positive for CDX2 in 34% of cases, and in clinically suspected BE in which goblet cells were not identified, the cardia-type mucosa was positive in 14%. Whether goblet cells were present in the same biopsy specimen or not, fundic-type mucosa was not labeled for CDX2.

One hypothesis proposes that the first step in the reflux-adenocarcinoma sequence is metaplasia from squamous to cardia-type mucosa.8,9 The metaplastic cardia mucosa has the potential to progress to IM or to oxyntic-cardia mucosa (so-called fundic type). Therefore, the expression of CDX2 reflects intestinal-type changes in the cardia mucosa, whereas the absence of CDX2 in fundic-type mucosa implies end differentiation of this type of mucosa along a pathway that has no neoplastic predisposition.

Villin seems to be a less specific marker of IM than CDX2 because it also stained fundic-type mucosa. However, in our hands, villin was more sensitive and its staining pattern more distinctive and identifiable than CDX2, enabling ready identification of IM. We, therefore, suggest the use of both markers.

HepPar-1 has been reported to be 100% positive in BE.2 It was negative in dysplastic glands and was only focally positive in 1 of 9 cases of adenocarcinoma.32 HepPar-1 used for the detection of IM in esophageal brush samples showed moderate sensitivity (82%) and did not stain cardia-type mucosa.33 Only 57% of our cases of BE were positive, and none of the cardia- or fundic-type mucosa expressed the antigen. HepPar-1 is, thus, of low sensitivity for the detection of IM in BE.

The separation of BE from IM of the gastric cardia was thought to be important by some investigators, and currently, patients with IM of the gastric cardia are not enrolled in surveillance. The distinction of the 2 entities is particularly difficult, and the existence of the cardia as a normal anatomic component of the stomach is disputed.34 It has been suggested that the presence of mucous glands in the gastroesophageal junction area represents an acquired form of metaplastic epithelium that develops as a result of GERD.8,9 A distinctive CK staining pattern of BE was initially reported. The so-called Barrett pattern was a diffuse, strong, full-thickness staining for CK7 and superficial staining for CK20.35 Subsequently, it was suggested that the staining patterns of CK7/CK20 in BE and IM arising from gastric cardia were different,36 but it soon became clear that these patterns were not consistent and were not diagnostic discriminators.37,38 The variations in reported results have been attributed to differences in methods and reagents and differences in the endoscopic and clinical definitions of Barrett metaplasia.39 We did not find CK7/CK20 expression to be particularly useful. The diffuse CK7 pattern of staining was present in 97% of the IM mucosa, 58% of the cardia-type mucosa, and none of the fundic-type mucosa. In cases without goblet cells, this pattern was observed in a higher proportion of cardia-type mucosa (79% [11/14]). Fundic-type mucosa did not show diffuse CK7 staining.

Recent studies of the epidemiology, prognosis, patterns of lymphatic metastasis, and survival for esophageal and gastroesophageal junction adenocarcinoma suggest that the tumors are similar.2,15 Furthermore, the rising rate of esophageal adenocarcinoma is paralleled by that of adenocarcinoma of the gastric cardia and by subcardial gastric carcinoma. A small prospective study of 93 patients with an initial diagnosis of IM of gastric cardia showed that gastric adenocarcinoma developed in 10 during the follow-up period, so that it was suggested that the risk of adenocarcinoma of the stomach in patients with IM may not be too different from the risk of adenocarcinoma of the esophagus in patients with BE.40 The nature of cardia-type mucosa in esophageal biopsy specimens is, thus, less relevant and we advocate that the patients with cardia-type mucosa, especially that expressing CDX2 or villin, should be included in surveillance for esophageal adenocarcinoma. The use of such markers in prospective studies will allow a means of validating the BSG statement that the presence of goblet cells is not a prerequisite for the diagnosis of IM which “can always be identified in endoscopically-visible columnar metaplasia providing a sufficient number of biopsies are taken over an adequate time-scale.”13

We propose that CDX2 and villin are sensitive markers for the detection of early IM and are suitable supplements for the histologic diagnosis of BE. HepPar-1 has limited sensitivity, and CK7 is not specific.

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