Overexpression of Human Carcinoma–Associated Antigen in Esophageal Adenocarcinoma and Its Precursor Lesions

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Abstract

The expression of human carcinoma–associated antigen (HCA), a mucin-type glycoprotein, was assessed in 50 esophagogastrectomy specimens. Areas, each from different cases, of Barrett esophagus (n = 36), low-grade dysplasia (n = 38), high-grade dysplasia/carcinoma in situ (n = 26), and esophageal adenocarcinoma (EAC; n = 34) were examined by immunohistochemical stains to 2 anti-HCA monoclonal antibodies, G1 and HAE3. These two antibodies showed similar staining patterns. HCA was overexpressed significantly in EAC and high-grade dysplasia/carcinoma in situ compared with benign esophageal mucosa (P < .001 for both), Barrett esophagus (P < .001 for both), and low-grade dysplasia (P < .025 for both). HCA overexpression did not correlate with the grade of EAC (P > .1). The results suggest that overexpression of HCA might help in diagnosing esophageal dysplasia and cancer. The correlation of HCA with the grade of esophageal dysplasia suggests its possible involvement in the pathogenesis of EAC. HCA also might provide a target for immunotherapy.

The incidence of esophageal adenocarcinoma (EAC) has risen dramatically in the United States and Europe during the past 3 decades, whereas the incidence of esophageal squamous cell carcinoma has remained stable.1,2 From 1976 to 1990, the incidence of EAC tripled in the United States, with an annual increase of approximately 10%.3 More than 13,900 new cases with 13,000 deaths were anticipated in the United States in 2003.4 Barrett esophagus is considered a key precancerous lesion that shows a strong association with the development of dysplasia and subsequent EAC.5 The mechanisms of such a process remain unclear. Early diagnosis is the best way to cure this deadly cancer.

Human carcinoma–associated antigen (HCA) is a mucin-type glycoprotein, which originally was identified by antibodies against epiglycanin purified from mouse mammary carcinoma cells.6,7 Two monoclonal antibodies against epiglycanin, G1 and HAE3, specifically bind HCA in vitro. The enzyme-linked immunosorbent assay has shown increased levels of HCA in the body fluids and serum samples of patients with a variety of carcinomas,8-10 indicating HCA potentially can be used to diagnose human carcinomas and to monitor the progression of these cancers. Furthermore, overexpression of HCA has been identified in prostatic adenocarcinoma11 and transitional cell carcinoma of the urinary bladder.12 In the present study, expression of HCA was assessed in EAC and its precursor lesions by using immunohistochemical analysis.

Materials and Methods

Selected from the surgical pathology files at Strong Memorial Hospital of University of Rochester Medical Center

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were 50 esophagogastrectomy specimens that contained the following areas in individual patients: Barrett esophagus, 36; low-grade dysplasia, 38; high-grade dysplasia/carcinoma in situ (CIS), 26; and EAC, 34 (well-differentiated, 7; moderately differentiated, 12; and poorly differentiated, 15). Two measures were taken to ensure the lesions were Barrett esophagus and not gastric cardia associated: (1) All cases were selected from the patients who had a history of Barrett esophagus and/or Barrett esophagus–associated problems. (2) Strict topographic criteria were used to select only the cases in which the main tumors and/or lesions were clearly in the esophagus.

Of the 50 patients, 12 were women and 38 were men. The patients’ ages ranged from 42 to 84 years (mean, 65.4 years). For 42 patients, follow-up data for 2 to 98 months (mean, 25.6 months) were available, and for 8 patients, no follow-up data were available.

Generation of the IgM monoclonal antibodies G1 and HAE3 and immunohistochemical staining were performed as previously described.12 Briefly, 5-µm-thick sections were deparaffinized, quenched with 3% hydrogen peroxide for 6 minutes, and heated 40 minutes at 95°C to 99°C in an antigen retrieval solution (citrate buffer, pH 6.1; DAKO, Carpinteria, CA) in a steamer (Black and Decker, Shelton, CT). The slides were stained for 60 minutes with G1 and HAE3 followed by 30-minute incubations each in goat antimouse IgM (Vector Laboratories, Burlingame, CA) and streptavidin–horseradish peroxidase (Jackson Laboratories, West Grove, PA). The positive reaction was visualized with the chromogen 3-amino-9-ethylcarbazole (DAKO).

The staining of 10% or more of the cells was considered positive and was graded as 1+ (weak), 2+ (strong), and 3+ (strongest) according to staining intensity. Overexpression of HCA was defined as 2+ staining or more. The Fisher exact test was used for statistical analysis.

Results

G1 and HAE3 showed variable amounts of granular staining in the cytoplasm, on the luminal surface, and in secreted mucin. Both antibodies showed similar staining results. However, HAE3 was more sensitive than G1, whereas G1 yielded less background staining.

Normal esophageal squamous or gastric glandular epithelial cells usually did not stain with G1 and HAE3, except for occasional focal cytoplasmic (in columnar cells) or membranous (in squamous cells) staining in the mucosa adjacent to cancer or dysplasia. In Barrett esophagus, the stain was predominantly in the cytoplasm of goblet cells. In dysplasia and EAC, the stain was diffusely cytoplasmic and luminal. Stromal staining was observed only in EAC.

The percentage of positive cells and staining intensity of G1 and HAE3 were proportional to the severity of the esophageal lesions. Overexpression of HCA was observed significantly more often in EAC and high-grade dysplasia/CIS than in benign esophageal mucosa (P < .001 for both), Barrett esophagus (P < .001 for both), and low-grade dysplasia (P < .025 for both) Table 1 and Image 1. Furthermore, low-grade dysplasia also showed significant overexpression of HCA compared with benign or Barrett esophagus (P < .05 for both). There was no significant difference of HCA overexpression between high-grade dysplasia/CIS and EAC (P = .21).

G1 and HAE3 stains tended to correlate with the grade of EAC Table 2. However, the difference in HCA overexpression among the well-, moderately, and poorly differentiated EAC was not statistically significant (P > .1).

Association of HCA overexpression in EAC with patient age, tumor size, clinical and pathologic stage, and survival time was analyzed with multivariate analysis. The results showed no independent correlation of HCA overexpression with those factors (data not shown).

Discussion

The incidence of EAC increased more rapidly than any other cancer in the United States between 1976 and 1990,3 but this cancer is one of the least studied and deadliest cancers in the world.4 Although it is known that Barrett esophagus can lead to the development of EAC through dysplasia,
Image II Expression of human carcinoma–associated antigen in benign, dysplastic, and malignant esophagus. A and B, Barrett esophagus (A, H&E, ×100; B, HAE3, ×100). C and D, Low-grade (right lower quadrant) and high-grade (left upper quadrant) dysplasia (C, H&E, ×100; D, HAE3, ×100). E and F, Esophageal adenocarcinoma (E, H&E, ×200; F, HAE3, ×200).
the mechanisms of such progression remain unclear. Overexpression of HCA in human cancers might provide a valuable tool not only to aid the early and accurate diagnosis of cancer but also to monitor the progression of cancer. Overexpression of HCA also might provide a new target for treatment.

Mucins proteins have been studied intensively during the last decade. The 9 types of mucin identified so far are divided into 2 classes: secreted only (MUC2, MUC5AC, MUC5B, MUC6, MUC7, and MUC8) and membrane-bound/secreted (MUC1, MUC3, and MUC4).13,14 Secreted mucins are produced by epithelial cells in many human organs and function in the lubrication, protection, and formation of a selective barrier on epithelial surfaces. The membrane-bound mucin also is present in nonepithelial tissue, and its function is not well understood. However, MUC1 is shown to be associated with cell-cell and cell–extracellular matrix interactions, lymphocyte trafficking, and the protection of cells against microorganisms.13,14 The molecular structure and function of HCA and its relationship to known mucin proteins are unknown. The presence of HCA predominantly in the cytoplasm and lumen suggests that HCA is a membrane-bound/secreted mucin, which is consistent with its detection in serum. HCA in the stroma within or around the invasive EAC most likely results from the diffusion from epithelial cell secretion.

In many human carcinomas, the mucin proteins are produced aberrantly, including an abnormal glycosylation pattern, expression pattern, and expression level.13,14 Tumor-associated mucins are underglycosylated. The expression patterns of certain mucins such as MUC1 also are changed from the apical cell membrane location in normal tissue to the entire cell membrane. The expression levels of mucins are variable in human cancers. MUC1 is the most extensively studied mucin. High levels of MUC1 have been detected in a variety of epithelial tumors such as breast and ovarian cancers.15-17 Moreover, an increased serum level of MUC1 is associated with poor survival of patients with breast cancer, carcinoma of the ampulla of Vater, and gastric carcinoma.18-20 MUC2 and MUC4 also are up-regulated in carcinomas of the lung, stomach, and pancreas.21-24 Overexpression of HCA in EAC vs Barrett esophagus or low-grade dysplasia together with an aberrant expression pattern of HCA in the lumen and stroma in this study suggest that HCA might serve as a valuable biomarker for the diagnosis of Barrett-related dysplasia and EAC.

The differential diagnosis of high-grade from low-grade dysplasia in Barrett esophagus is a critical issue in pathology because high-grade dysplasia of Barrett esophagus might result in an esophagectomy. Many biomarkers have been tested to help define high-grade dysplasia, but none of them has been proven diagnostic.25-28 The significant overexpression of HCA in high-grade dysplasia/CIS vs low-grade dysplasia rising in Barrett esophagus has provided one more clue to help with the diagnosis of high-grade dysplasia.

Many genes, including oncogenes, tumor suppressor genes, growth factors and their receptors, apoptosis genes, DNA mismatch repair genes, and the p53 gene show aberrant transcription and translation in EAC.5,29 It is known that EAC can results from the sequence of metaplasia-dysplasia-carcinoma, ie, Barrett esophagus to low-grade dysplasia to high-grade dysplasia to EAC. However, the molecular mechanisms in such a process remain unclear. This study demonstrates a correlation between overexpression of HCA and the progressive severity of Barrett-associated lesions, indicating that HCA might be involved in the progression of dysplasia and the subsequent development of EAC.

Multivariate analysis did not show any correlation of HCA overexpression in EAC with prognosis or other clinicopathologic factors. Such negative results could be the result of a small sample and suboptimal follow-up data. Only 34 cases of EAC were included in the study, and the sample becomes even smaller when divided into groups with and without overexpression of HCA. Follow-up data were obtained from the patients’ medical records in our hospital, and the follow-up period varied considerably, from 2 to 98 months. It might not represent the actual survival time.

Immunotherapy with mucin monoclonal antibodies has been exploited in carcinomas of the bladder, lung, and breast with promising results.13 Bladder tumors are particularly interesting, owing to possible intravesical delivery of therapeutic agents, which might dramatically reduce systemic side effects. Because the esophagus has the same advantage of easy accessibility for local application of treatment agents as...
the urinary bladder, and high-grade dysplasia/CIS and EAC showed significant overexpression of HCA, monoclonal antibodies (HAE3 and G1) directed against HCA might serve as potential immunotherapeutic agents.

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References