

Expression of the High Mobility Group Proteins HMGI(Y) Correlates with Malignant Progression in Barrett's Metaplasia

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Abstract

Expression of the high mobility group proteins HMGI(Y) has been shown to be a marker of malignancy in thyroid and pancreatic lesions and to correlate significantly with malignant progression in the colon. The aim of this study was to determine whether HMGI(Y) expression is associated with malignant progression in Barrett's metaplasia (BM). Immunoperoxidase staining for HMGI(Y) was performed on sections of formalin-fixed paraffin-embedded endoscopic esophageal biopsies from 42 patients with BM. These consisted of 19 biopsies negative for dysplasia (ND), 16 with low-grade dysplasia (LGD)/indeterminate for dysplasia (IND), and 7 with high-grade dysplasia (HGD)/adenocarcinoma (CA). The percentage of positive cells was recorded, and nuclear HMGI(Y) immunoreactivity in >10% of the cells was considered positive. Statistical analysis was performed using Fisher's exact test. Positive HMGI(Y) staining was detected in 2 of 19 (11%) cases ND, 5 of 16 (30%) LGD/IND cases, and 7 of 7 (100%) HGD/CA cases. Biopsies with HGD/CA were significantly more likely to be positive for HMGI(Y) than biopsies ND ($P < 0.0001$) or with LGD/IND ($P = 0.0046$). We conclude that HMGI(Y) expression is significantly associated with malignant progression in BM. Additional studies are needed to determine whether BM biopsies that are ND or LGD/IND and positive for HMGI(Y) are more likely to progress to adenocarcinoma.

Introduction

The significant inter- and intraobserver variability in the morphological grading of dysplasia in Barrett's metaplasia (BM) is well known. Objective markers of malignant progression are needed so that cancer risk in patients with BM can be assessed more accurately. The high mobility group (HMG) proteins are small nonhi-

stone chromosomal binding proteins, involved in organizing the chromatin structure (1). HMG-I and HMG-Y proteins are encoded by the same gene, *HMGI(Y)*, by alternately spliced mRNA (2, 3). HMG-C is encoded by a different genes (4). HMGI(Y) proteins can increase transcription by antagonizing histone H1-mediated general repression of transcription (5) and play an important role in transcription regulation through other specific pathways (6–14). Recent evidence suggests that these proteins may possess oncogenic properties (15, 16).

HMGI(Y) expression was found to correlate with malignant transformation (17–20). Using immunohistochemical staining, these proteins were found to be expressed in several human malignancies including colorectal (21–23), thyroid (24, 25), and pancreatic (26) carcinomas. More importantly, HMGI(Y) expression was detected early and with increasing frequency during malignant progression in human cervical (27) and colorectal (21, 22, 28) carcinogenesis.

The aim of this study was to determine whether HMGI(Y) expression is an early event during malignant progression in BM.

Materials and Methods

Tissues. H&E-stained tissue sections of formalin-fixed and paraffin-embedded biopsies from patients with BM enrolled in a prospective study in our institution were evaluated for dysplasia by two experienced gastrointestinal pathologists using previously published criteria (29). Because of the well known inter- and intraobserver variability in the grading of dysplasia, especially in distinguishing low-grade dysplasia (LGD) from indefinite/indeterminate (IND) and high-grade dysplasia (HGD) from adenocarcinoma (CA), cases with a diagnosis of LGD or IND were grouped in one group (LGD/IND), and those with HGD or CA were grouped in another group (HGD/CA), as proposed by Reid *et al.* (30). Nineteen cases negative for dysplasia (ND), 16 with LGD/IND, and 7 with HGD/CA were randomly selected for this study. All of the patients are receiving proton pump inhibitor therapy, as usually given to patients with BM, and none were taking part in any investigational treatment.

Antibody. Rabbit polyclonal antibody was generated against a 12-amino acid synthetic peptide corresponding to the NH₂ termini of human HMG-I and HMG-Y according to the sequences published previously by Johnson *et al.* (3). This peptide is identical to the peptide used to generate the antibody used in immunohistochemical study by other investigators (21, 23, 24, 27).

The peptide synthesis and antibody production were performed at a commercial facility (Research Genetics, Huntsville, AL). The antibody was affinity purified using the immunizing peptide, quantitated, and tested by ELISA at another commercial facility (Bethyl Laboratories, Montgomery, TX).

Western Blot Analysis. Whole cell lysates of the hepatocellular carcinoma cell line HepG2, which express HMGI(Y) (31), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Fifty- μ g aliquots were loaded on 12% SDS-polyacryl-

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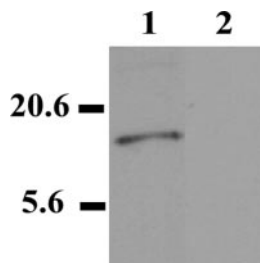


Fig. 1. Western blot analysis showing detection of HMGI(Y) protein in whole cell lysate of the human hepatoma cell line HepG2. The affinity-purified antibody APY96 detects a low molecular weight protein (Lane 1), which is not detected by a nonrelevant control antibody (Lane 2).

amide gel. After electrophoretic separation, the proteins were transferred onto nitrocellulose membranes. After blocking of nonspecific activity, membranes were incubated with either a 1:1000 dilution of the HMGI(Y) antibody APIY96 or a 1:1000 dilution of a nonrelevant affinity-purified rabbit antibody (negative control). The bound antibody was detected using a chemiluminescence detection kit and then exposed to radiographic film.

Immunohistochemical Staining. The immunoperoxidase staining was performed using a Dako automated immunostainer (Dako, Carpinteria, CA). Sections of formalin-fixed and paraffin-embedded HepG2 cells, prepared in our laboratory, were first used to optimize the immunohistochemical staining conditions. Several methods of antigen retrieval and antibody dilutions and incubation times were tested and compared, and then the optimal methods were used on the human tissue samples.

Sections of formalin-fixed and paraffin-embedded BM tissue from 19 biopsies ND, 16 with LGD/IND, and 7 with HGD/CA were subjected to heat-induced antigen retrieval in 10 mM citrate buffer in a pressure cooker for 5 min, followed by 15 min cooling off at room temperature. Sections were then washed and incubated for 20 min at room temperature with the affinity-purified antibody at 1:200 dilution. The bound antibody was detected using an Envision Plus Rabbit Peroxidase kit (Dako), with 3,3'-diaminobenzidine as chromogen. Sections were counterstained in hematoxylin, dehydrated, mounted, and coverslipped. Sections of formalin-fixed and paraffin-embedded HepG2 cell line were used as positive controls, and negative controls consisted of sections incubated without primary antibody or with an irrelevant antibody of the same animal species (rabbit).

The percentage of positive cells in each case was scored by one observer, who was blinded to the results of dysplasia grading.

Statistical Analysis. Statistical analysis was performed using Fisher's exact test using InStat statistical software, version 3.0a (GraphPad Inc., San Diego, CA).

Results

Western blot analysis shows that the antibody APIY96 detects a low molecular weight protein in HepG2 cells, corresponding to HMGI(Y), which is not detected in the control lane using a different nonrelevant antibody from the same species (Fig. 1).

By immunohistochemical staining, HMGI(Y) immunoreactivity was always nuclear (Fig. 2).

Nuclear HMGI(Y) immunoreactivity in >10% of the cells was considered positive. Although most cases of BM that were

ND were completely negative for HMGI(Y), 2 of the 19 (11%) cases were positive, showing immunoreactivity localized mainly in the surface epithelial cells. By contrast, positive HMGI(Y) immunostaining was present in 5 of 16 (30%) cases of BM with LGD/IND and in all 7 of 7 (100%) cases with HGD/CA. Significantly more cases of BM with HGD/CA were positive for HMGI(Y) than ND ($P < 0.0001$) or LGD/IND ($P = 0.0046$).

Discussion

The incidence of adenocarcinoma of the esophagus has been rising in the United States and Western Europe since the 1970s, and survival of this tumor, although improving, continues to be poor (32). The precursor lesion of this cancer is BM. Patients with BM are enrolled in surveillance programs aimed at detecting this cancer at an early stage, when surgical resection offers the best chance of survival (33). Surveillance programs are based on morphological assessment of dysplasia grade in histological sections of esophageal biopsies, with follow-up intervals largely dependent on the grade of dysplasia. However, there is significant intra- and interobserver variability in the grading of dysplasia in BM even among recognized gastrointestinal pathologists (34, 35). This variability can be substantial [a case called negative for dysplasia by one experienced pathologist and was called high-grade dysplasia by another (35)]. In a recent study, 16 cases diagnosed initially as LGD were reviewed by three experienced pathologists. At least one of the three confirmed a diagnosis of LGD in all 16 cases, but all three agreed on a diagnosis of LGD in only 4 of the 16 cases (25%; Ref. 36), highlighting the subjectivity of such diagnosis. In addition, using dysplasia grading as the sole marker of malignant progression in BM presents another problem. There are two extremes (ND, usually a starting point, and HGD/CA, usually an end point with esophagectomy performed on most patients), and there is only one intermediate marker, LGD/IND. When an individual is diagnosed with LGD/IND on the first biopsy, the gastroenterologist has no idea how long the patient has had LGD/IND or, more importantly, how long it will be before the patient develops HGD/CA. An objective marker or, preferably, several objective markers are needed to improve the effectiveness and cost-effectiveness of surveillance programs for patients with BM. Ideally, these would include early, intermediate, and late markers that lie in between ND and HGD/CA and would be far more helpful in designing cost-effective surveillance programs. Moreover, they would serve as surrogate end point markers in chemoprevention trials.

We have previously proposed p53 protein accumulation, detected by immunoperoxidase staining, as a marker of malignant progression in BM (37, 38), which was subsequently confirmed by others (36, 39, 40). In the present study, we show that HMGI(Y) expression correlates with malignant progression in BM. Whereas 0 of 36 patients ND (0%) was positive for p53 in our previous study (38), 11% of the cases ND are positive for HMGI(Y) in the present study. We speculate that HMGI(Y) may be expressed earlier than p53 during malignant progression in BM. Additional studies are needed to determine (a) whether HMGI(Y) expression in BM biopsies ND or those with LGD/IND predicts malignant progression and (b) whether HMGI(Y) is expressed earlier than p53 during malignant progression in BM. Such studies are currently in progress in our laboratory.

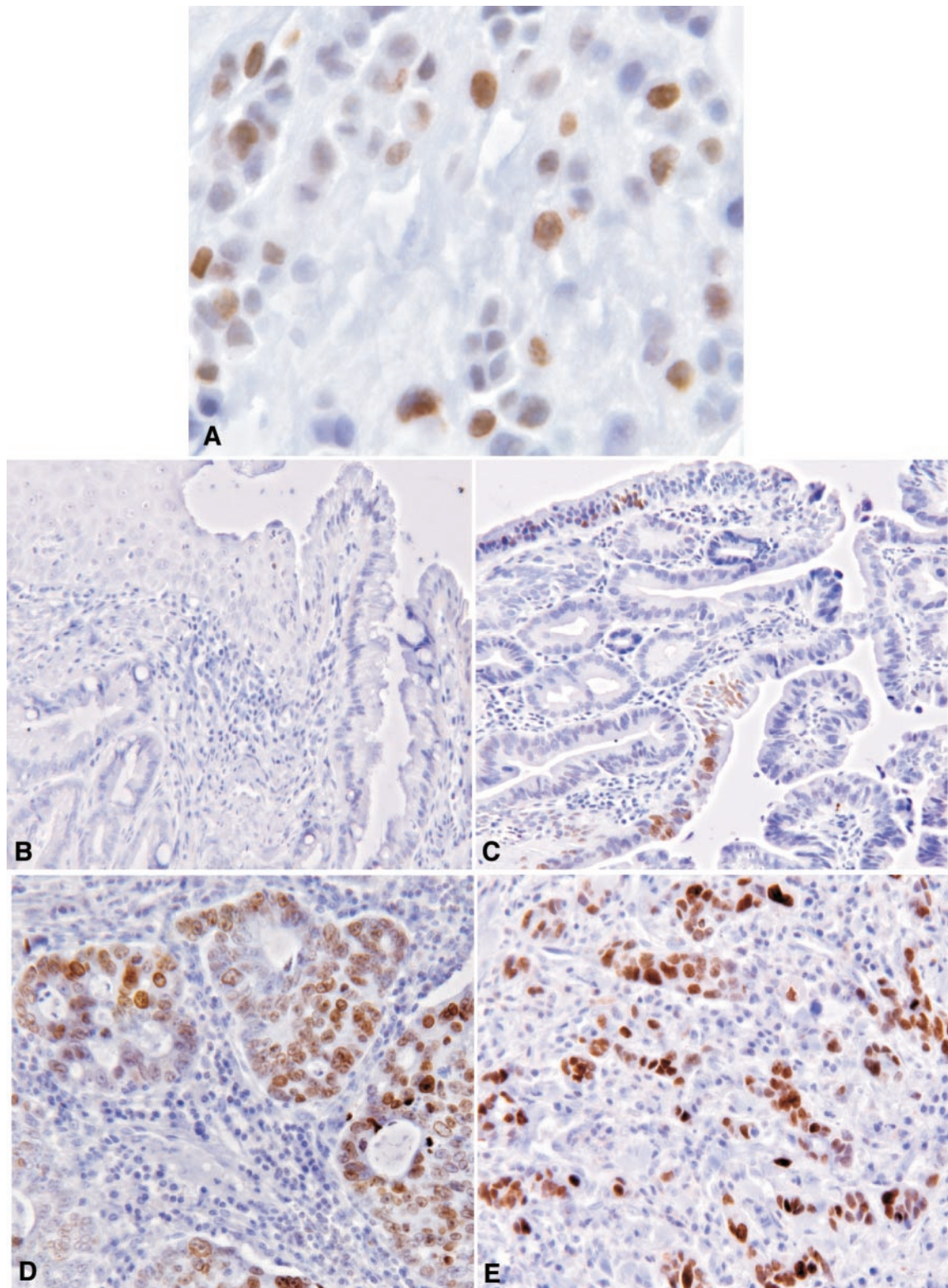


Fig. 2. HMGI(Y) expression in Barrett's metaplasia (BM). The positive control cell line, HepG2, shows strong nuclear immunoreactivity (A). Although a few cases of BM negative for dysplasia were HMGI(Y) negative, the majority of BM negative for dysplasia cases were negative for HMGI(Y) as in this example (B). Nuclear HMGI(Y) immunoreactivity is present in cases of BM with low-grade dysplasia/indeterminate for dysplasia (C) and in moderately differentiated (D) and poorly differentiated (E) adenocarcinoma. Panels represent immunoperoxidase staining with hematoxylin counterstaining.

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