Prediction of response to endoscopic therapy of Barrett's dysplasia by using genetic biomarkers CME

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Background: Endoscopic therapy for the treatment of high-grade dysplasia (HGD) and intramucosal cancer (IMC) in Barrett's esophagus (BE) may not always result in complete remission of dysplasia (CRD).

Objective: To determine whether genetic alterations in the Barrett's mucosa can predict response to endoscopic therapy.

Design: Retrospective cohort study.

Setting: Tertiary-care institution.

Patients: Selected patients who underwent endoscopic therapy for BE containing HGD/IMC between 2003 and 2010.

Interventions: Endoscopic therapy combining mucosal resection and different ablation modalities was performed based on patient characteristics, endoscopic findings, and technique evolution. Fluorescence in situ hybridization was used to evaluate genetic alterations on baseline endoscopic cytology brushings by using probes directed to loci 8q24 (*MYC*), 9p21 (*CDKN2A; alias P16*), 17q12 (*ERBB2; alias Her-2/neu*), and 20q13.2 (*ZNF217*).

Main Outcome Measurements: Genetic biomarkers predicting achievement of CRD after endoscopic therapy.

Results: A total of 181 patients were included (145 men; 66 ± 10 years of age). There were 130 patients (72%) who responded to endoscopic therapy with CRD. Multiple gains detected by fluorescence in situ hybridization was found to be a negative predictor (hazard ratio 0.57; 95% confidence interval, 0.40-0.82) after adjusting for potential clinical confounders. Similar results were found when analyses were restricted to patients (n = 66) undergoing radiofrequency ablation (hazard ratio 0.58; 95% confidence interval, 0.31-1.09).

Limitations: Retrospective study, heterogeneity of treatment modalities.

Conclusion: Patients with multiple gains detected by brush cytology specimens may have a lower response rate to endoscopic therapy. The presence of multiple gains can be an adjunct to standard histology in prognosticating BE patients with HGD/IMC undergoing endoscopic therapy. (Gastrointest Endosc 2014;80:984-91.)

High-grade dysplasia (HGD) and intramucosal cancer (IMC) associated with Barrett's esophagus (BE) carry a high risk of progression to esophageal adenocarcinoma (EAC).[1,2] Endoscopic therapies including EMR and radiofrequency ablation (RFA) have been shown to be effective for the treatment of HGD and IMC and are less-invasive

Abbreviations: BE, Barrett's esophagus; BMI, body mass index; CRD, complete remission of dysplasia; EAC, esophageal adenocarcinoma; FISH, fluorescence in situ hybridization; HGD, high-grade dysplasia; HR, hazard ratio; IMC, intramucosal cancer; IQR, interquartile range; PDT, photodynamic therapy; RFA, radiofrequency ablation; SLG, single locus gain.

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alternatives to conventional esophagectomy.[3–5] Despite initial response to endoscopic therapy with downstaging of dysplasia, a significant number of patients will not achieve complete remission of dysplasia (CRD).[6–8] This has intensified the interest in whether biomarkers can help to predict outcome after endoscopic therapy.

The majority of EACs associated with BE exhibit chromosomal abnormalities.[9,10] In addition, a number of chromosomal alterations have been associated with an increased risk of progression from nondysplastic BE and low-grade dysplasia to HGD or EAC. Among them are several markers of chromosomal instability including DNA content abnormalities such as aneuploidy (ie, a cell containing either extra or missing copies of particular chromosomes), loss or inactivation of tumor suppressor genes (eg, *P53*, *P16*), and amplification of oncogenes (eg, *Her-2/neu, MYC)*.[11–17] It has been shown that these genetic alterations can be found in large areas of the Barrett's mucosa, even in areas without morphological features of dysplasia.[18,19] Hage et al[20] investigated the effect of ablation therapy on molecular abnormalities (cellular proliferation, aneuploidy, and p53 overexpression) in the Barrett's mucosa and found that they were still present in a subset of patients with persistent Barrett's epithelium, despite intensive ablation with argon plasma coagulation and photodynamic therapy (PDT). In a longitudinal case series of 19 patients undergoing RFA, somatic mutations (ie, *P16*, *P53*) were evaluated pre- and post-ablation therapy. Five patients demonstrated persistence of HGD/IMC as well as persistent mutations post-ablation, suggesting that persistence of genetic abnormalities may be associated with persistent pathology.[21] However, few studies have investigated the ability of genetic markers to predict the outcome after endoscopic therapy.[22,23] If biomarkers can be identified that can predict outcome after endoscopic treatment, this may help to optimize both treatment and surveillance strategies.

Fluorescence in situ hybridization (FISH) is a technique that can be applied to cytology specimens obtained by easily collected endoscopic brushings. It uses fluorescently labeled DNA probes that hybridize to multiple chromosomal locations. Abnormal gains or losses of chromosomes, chromosomal regions, or specific chromosomal loci can be visualized with a fluorescence microscope. We have found that the use of cytological specimens are more likely representative of the entire Barrett's mucosa and are less subject to sampling bias as biopsy specimens. Our multitarget FISH assay consisting of locus-specific probes to 8q24 (*MYC*), 9p21 (*CDKN2A; alias P16*), 17q12 (*ERBB2; alias Her-2/neu*), and 20q13.2 (*ZNF217*) can be used to detect gains, amplification, or deletions of 4 genes that are frequently altered during neoplastic progression in BE.[10,24] Gains of multiple probes is an indicator of increasing chromosomal instability and has been associated with advanced stages of dysplasia as well as with an increased risk of neoplastic progression.[10]

Take-home Message

- Genetic biomarkers such as those assessed with fluorescence in situ hybridization could be an adjunct to standard histology in predicting which patients with Barrett's dysplasia will respond to endoscopic therapy.
- Patients with multiple gains detected by brush cytology specimens had a lower response rate to endoscopic therapy.

Previously, we demonstrated that allelic loss of *P16* detected by FISH is a predictor of a decreased response in patients undergoing PDT for Barrett's dysplasia.[23] At present, a variety of endoscopic techniques including endoscopic resection and ablation techniques are used for the treatment of Barrett's dysplasia. The aim of this study was to determine the predictive value of genetic biomarkers in BE patients undergoing multiple-mode endoscopic therapy. Recently, RFA has become the standard of care for patients with HGD because of the increasing evidence of its efficacy and its favorable side-effect profile. Therefore, in addition, we performed a subanalysis of patients who underwent RFA.

METHODS

Study design

This was a retrospective cohort study. Medical records of all BE patients who had undergone endoscopic therapy at our tertiary referral center between April 2003 and December 2010 as well as having cytology obtained for FISH analysis were reviewed. This analysis was performed with approval from the Institutional Review Board of the Mayo Clinic.

Patients

We included BE patients with histologically confirmed HGD or IMC who underwent endoscopic therapy with or without preceding EMR in case of any visible lesions. BE was defined as the presence of a columnar-lined distal esophagus, visible as pink mucosa extending above the top of the gastric folds during endoscopy confirmed by the presence of specialized intestinal metaplasia on biopsy specimens. Inclusion was limited to (1) patients in whom endoscopic brushing specimens used for FISH analysis were obtained up to 3 months before endoscopic therapy and (2) availability of biopsy results after endoscopic treatment. Patients with signs of lymph node metastasis or distant metastasis on EUS or CT scan were excluded from the study as well as patients who were diagnosed with invasive EAC during the initial treatment endoscopy. Relevant clinical and endoscopic data were collected from a prospectively maintained database of electronic medical records, and FISH results were obtained by review of our FISH database. Extracted data included age, sex,