2015 JMD CME Program

The Journal of Molecular Diagnostics, Vol. 17, No. 4, July 2015

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

Evaluation of Mutational Testing of Preneoplastic Barrett's Mucosa by Next-Generation Sequencing of Formalin-Fixed, Paraffin-Embedded Endoscopic Samples for Detection of Concurrent Dysplasia and Adenocarcinoma in Barrett's Esophagus

Armando Del Portillo,* Stephen M. Lagana,* Yuan Yao,† Takeshi Uehara,‡ Nirag Jhala,† Tapan Ganguly,§ Peter Nagy,* Jorge Gutierrez,* Aesis Luna,* Julian Abrams,¶ Yang Liu,‖ Randall Brand,‖ Jorge L. Sepulveda,* Gary W. Falk,** and Antonia R. Sepulveda*

From the Department of Pathology and Cell Biology and the Division of Gastroenterology,¶ Columbia University, New York City, New York; the Departments of Pathology and Laboratory Medicine† and Genetics§ and the Division of Gastroenterology,** University of Pennsylvania, Philadelphia, Pennsylvania; the Department of Pathology,‡ Shinshu University, Nagano, Japan; and the Department of Medicine,‖ University of Pittsburgh, Pittsburgh, Pennsylvania*

CME Accreditation Statement: This activity ("JMD 2015 CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity ("JMD 2015 CME Program in Molecular Diagnostics") for a maximum of 36 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

CME Disclosures: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication February 24, 2015.

Address correspondence to Antonia R. Sepulveda, M.D., Ph.D., Department of Pathology and Cell Biology, Columbia University, 630 W. 168th St., VC-14, Room 212, New York, NY 10032. E-mail: as4400@columbia.edu.

Barrett's intestinal metaplasia (BIM) may harbor genomic mutations before the histologic appearance of dysplasia and cancer and requires frequent surveillance. We explored next-generation sequencing to detect mutations with the analytical sensitivity required to predict concurrent high-grade dysplasia (HGD) and esophageal adenocarcinoma (EAC) in patients with Barrett's esophagus by testing nonneoplastic BIM. Formalin-fixed, paraffin-embedded (FFPE) routine biopsy or endoscopic mucosal resection samples from 32 patients were tested: nonprogressors to HGD or EAC (BIM-NP) with BIM, who never had a diagnosis of dysplasia or EAC ($N = 13$); progressors to HGD or EAC (BIM-P) with BIM and a worse diagnosis of HGD or EAC ($N = 15$); and four BIM-negative samples. No mutations were detected in the BIM-NP (0 of 13) or BIM-negative samples, whereas the BIM-P samples had mutations in 6 (75%) of 8 cases in *TP53, APC,* and *CDKN2A* ($P = 0.0005$), detected in samples with as low as 20% BIM. We found that next-generation sequencing from routine FFPE nonneoplastic Barrett's esophagus samples can detect multiple mutations in minute areas of BIM with high analytical sensitivity. Next-generation sequencing panels for detection of *TP53* and possibly combined mutations in other genes, such as *APC* and *CDKN2A,* may be useful in the clinical setting to improve dysplasia and cancer surveillance in patients with Barrett's esophagus. *(J Mol Diagn 2015, 17: 412—419; http://dx.doi.org/10.1016/j.jmoldx.2015.02.006)*

Supported in part by a Clinical and Translational Research Institute-Pilots Across the Spirit Program (CTSI-PATS) pilot grant from the Institute for Translational Medicine and Therapeutics (University of Pennsylvania, Philadelphia, PA; A.R.S. and G.W.F.); the Department of Pathology and Cell Biology (Columbia University, New York City, NY); pilot award 5U54CA163004 from the BETRNET consortium (A.R.S.); and NIH grants UL1TR000005 (Y.L. and R.B.), R01EB016657 (Y.L.), and R01CA185363 (Y.L.).

Disclosures: None declared.

http://dx.doi.org/10.1016/j.jmoldx.2015.02.006

Esophageal adenocarcinoma (EAC) most frequently develops in patients with Barrett's esophagus (BE), estimated to affect 3.3 million adults in the United States.[1] BE results from injury of the esophageal mucosa associated with gastroesophageal reflux, which leads to esophagitis and eventually BE. The incidence of EAC has increased greater than fivefold during the past 4 decades in the United States, paralleling the increase in detection of esophageal reflux and diagnosis of BE.[2] Barrett's intestinal metaplasia (BIM) is characterized by the replacement of normal squamous esophageal mucosa by columnar epithelium with intestinal metaplasia, often occurring in the background of patches of cardiac, oxyntic, or cardiooxyntic mucosa along the length of the BE. Patients with BE may sequentially progress to low-grade dysplasia, high-grade dysplasia (HGD), and eventually EAC. Patients with BE without dysplasia have a lower EAC risk (0.1% to 0.5% per patient-year) than those with high-grade dysplasia (6% to 19% per patient-year).[3,4] Current guidelines for the prevention of EAC require repeat surveillance endoscopies with biopsies of the Barrett's mucosa followed by pathological examination to detect BIM and dysplasia.[4–6] Unfortunately, the detection of dysplasia is hampered by sampling errors and high interobserver diagnostic variability.[7–10]

Known risk factors associated with EAC include male sex, older age, white race, hiatal hernia size, length of Barrett's epithelium, smoking, and high body mass index.[6] Recently, it was reported that persistence of BE negative for dysplasia over several endoscopic examinations identifies patients who are at low risk for development of EAC.[11]

It has been hoped that surveillance could potentially be improved by the implementation of risk stratification protocols using both clinical and biological markers because management of BE is costly and inefficient because only a small percentage of patients with BE progress to HGD or EAC.[12] Furthermore, recent data revealed that endoscopic surveillance of patients with BE was not associated with a substantially decreased risk of death from EAC.[13]

Biomarkers that can be assessed in random biopsy specimens from Barrett's mucosa negative for dysplasia with the ability to predict development or concurrent (co-existing) dysplasia elsewhere in the esophagus are warranted to improve surveillance approaches in the BE population. Previously evaluated testing approaches for EAC risk stratification in BE using esophageal biopsy samples include nuclear DNA content abnormalities, such as aneuploidy and tetraploidy; gene copy number alterations, such as loss of heterozygosity of p16 (*CDKN2A*) and p53 (*TP53*)[14,15]; somatic gene mutations; and hypermethylation of a number of genes.[14,16,17] However, published studies used fresh or frozen tissue or special sampling procedures, which limits their clinical implementation, and data from studies using routine formalin-fixed, paraffin-embedded (FFPE) clinical endoscopic samples replicating the clinical setting of BE patients undergoing endoscopic surveillance have not been reported.[6]

Next-generation sequencing (NGS) approaches using nucleic acids obtained from routinely processed FFPE tissues to detect mutations in cancer samples, enabling high analytical sensitivity and detection of low-frequency mutational events, are well established. However, NGS testing of preneoplastic tissues, such as BE, have not been evaluated in routine FFPE clinical samples, and there is no information regarding the minimal percentage of intestinal metaplasis in the tested mucosal tissue that may be considered for mutation testing. Therefore, we hypothesized that targeted NGS may be ideally suited for clinical application in the BE surveillance setting because it can be performed with minimal amounts of DNA from routine FFPE tissue samples, permits biomarker multiplexing, and can reach high sensitivity to detect low-frequency mutational events in heterogeneous BE tissues, where the foci of intestinal metaplasia may be small. Sensitivity of mutation detection by NGS can be 0.5% or lower due to the high level of sequencing coverage, reaching several thousand reads per amplicon when targeted sequencing is used. Therefore, targeted NGS of BE FFPE samples enables the characterization of the mutational status of hundreds to thousands of target functional sites in oncogenes and tumor suppressor genes that may be critical in BE, dysplastic precursor lesions, and EAC in individual biopsy samples collected in the clinical diagnostic setting.

We used two NGS-targeted amplicon sequencing technology platforms, the Illumina (San Diego, CA) TruSeq Cancer Panel for Illumina MiSeq platform and the Ion Torrent Ion AmpliSeq Cancer Panel (Life Technologies, Carlsbad, CA), to determine whether mutations of genes known to undergo mutagenesis in esophageal dysplasia or cancer arising in BE could predict the presence of HGD or EAC through testing random nondysplastic or noncancer BE mucosa with intestinal metaplasia using endoscopic FFPE samples.

Materials and Methods

Patients and Tissue Samples

Patients with esophageal biopsies or endoscopic mucosal resections (EMRs) with the pathological diagnosis of intestinal metaplasia, HGD, or EAC were searched in the pathology records from the University of Pennsylvania and Columbia University. The EAC lesions were intramucosal or superficial submucosal adenocarcinomas. Two patient groups were selected: nonprogressors to HGD or EAC (BIM-NP) are patients with a histologic diagnosis of intestinal metaplasia in at least two biopsies within $\geq$2 consecutive years and who never had a diagnosis of dysplasia (indefinite, low grade, or high grade) or EAC in any esophageal biopsy or resection, and progressors to HDG or EAC (BIM-P) are patients with a histologic diagnosis of intestinal metaplasia with a concurrent worse diagnosis of HGD or EAC. BIM-NP patients had a mean of