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Metabolomic Evidence for a Field Effect in Histologically Normal and Metaplastic Tissues in Patients with Esophageal Adenocarcinoma[1]

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Abstract

Patients with Barrett's esophagus (BO) are at increased risk of developing esophageal adenocarcinoma (EAC). Most Barrett's patients, however, do not develop EAC, and there is a need for markers that can identify those most at risk. This study aimed to see if a metabolic signature associated with the development of EAC existed. For this, tissue extracts from patients with EAC, BO, and normal esophagus were analyzed using ^{1}H nuclear magnetic resonance. Where possible, adjacent histologically normal tissues were sampled in those with EAC and BO. The study included 46 patients with EAC, 7 patients with BO, and 68 controls who underwent endoscopy for dyspeptic symptoms with normal appearances. Within the cancer cohort, 9 patients had nonneoplastic Barrett's adjacent to the cancer suitable for biopsy. It was possible to distinguish between histologically normal, BO, and EAC tissue in EAC patients [area under the receiver operator curve (AUROC) 1.00, 0.86, and 0.91] and between histologically benign BO in the presence and absence of EAC (AUROC 0.79). In both these cases, sample numbers limited the power of the models. Comparison of histologically normal tissue proximal to EAC versus that from controls (AUROC 1.00) suggests a strong field effect which may develop prior to overt EAC and hence be useful for identifying patients at high risk of developing EAC. Excellent sensitivity and specificity were found for this model to distinguish histologically normal squamous esophageal mucosa in EAC patients and healthy controls, with 8 metabolites being very significantly altered. This may have potential diagnostic value if a molecular signature can detect tissue from which neoplasms subsequently arise.

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Introduction

In many Western countries, rates of esophageal adenocarcinoma (EAC) have been increasing for more than 20 years, particularly among overweight, white men and those with severe gastroesophageal reflux disease [1,2]. Among patients with gastroesophageal reflux disease, some develop Barrett's esophagus (BO), characterized by metaplastic columnar epithelium in which mucus-secreting goblet cells appear. In some patients, this lining becomes unstable, progressing from low-grade to high-grade dysplasia (HGD) and then neoplasia. Identification of this at-risk population presently relies on endoscopic surveillance of large cohorts of patients with BO, most of whom will not develop a cancer.

The exact risk of patients with BO and HGD developing EAC is not known, but one meta-analysis gave a weighted incidence rate of 6.58 per 100 patient-years during the first 1.5 to 7 years [3].

Abbreviations: EAC, esophageal adenocarcinoma; BO, Barrett's esophagus; HGD, high-grade dysplasia; NMR, nuclear magnetic resonance; PCA, principle component analysis; PLS-DA, partial least squares discriminant analysis; ML-PLS-DA, multilevel partial least squares discriminant analysis; AUROC, area under the receiver operator curve.

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Likewise, Konda et al. suggested that the true rate of invasive EAC was 12% in patients diagnosed with HGD who underwent surgical resection. The other 88% of patients had only HGD or intramucosal carcinoma, potentially treatable by endoscopic ablation or endoscopic mucosal resection [4,5]. New markers are needed to distinguish BO patients at highest risk of developing EAC and to guide treatment options [6]. Identifying patients with BO and progression to HGD based on histology alone can be challenging because of sampling limitations and interobserver variability among pathologists [7]. In addition, most endoscopic studies have focused on the Barrett's epithelium itself, with little attention given to the squamous epithelium.

The presence of genetic mutations and evidence of dysregulation in histologically unaffected tissues adjacent to cancers implies a "field effect" that might be exploited if signatures exist that are associated with progression to HGD and intramucosal cancer in BO patients. [8]. Many different field effect biomarkers including changes in gene and protein expression, and epigenetic and metabolomic markers have been reported for different types of cancers [9]. Different techniques have been used to detect field effects in EAC, including nanoscale structural properties [10,11] and nuclear magnetic resonance (NMR)–based metabolomics of histologically normal cells proximal to EAC [12].

Some of the previous EAC metabolomics studies were based on different types of samples, relying on serum or urine samples to separate EAC patients from normal or other cancer patients. Sanchez-Espiridian et al. identified a panel of possible serum biomarkers to distinguish EAC patients and healthy controls using a liquid chromatography/mass spectrometry (MS) apprEACh for samples from more than 650 patients and controls [13]. Likewise, Davis et al. used ^{1}H-NMR metabolomics on urine samples to distinguish EAC patients or Barrett's patients from controls. Ikeda et al. used gas chromatography/MS metabolomics on human serum to identify various different biomarkers that distinguished EAC patients from colon cancer patients, gastric cancer patients, and controls [14]. Zhang et al. used liquid chromatography/MS and NMR to build a model based on samples from cancer patients and controls to address the more challenging task of separating EAC patients from patients with BO and HGD using serum metabolite levels [15].

There have also been two tissue metabolomics studies on EAC. Yakoub et al. [12] reported that a high phosphocholine/glutamate ratio indicated the presence of cancer proximal to histologically normal tissue in a ^{1}H Magic Angle Spinning NMR study of 35 EAC patients and 52 controls. Doran et al. observed a decrease in the ratio of carbohydrate to creatine-containing metabolites in Barrett's tissue samples in the presence of EAC compared with Barrett's in the absence of EAC for 29 controls and 43 cancer patients [16].

The present investigation was carried out using ^{1}H-NMR spectroscopy–based metabolomics looking at tissue samples from patients with EAC, patients with BO, or controls. The same samples were also subject to a previous Matrix-Assisted Laser Desorption/Ionisation analysis [17]. Metabolic profiling from both squamous and columnar epithelia across a range of patients was undertaken. The goal of this study was to identify specific metabolic profiles in EAC tissues compared to BO and control tissues, including metabolic changes in the histologically nonneoplastic tissues adjacent to EAC. This study attempted to identify metabolic markers that identify EAC and see if there was evidence of a field effect in histologically normal squamous or nondysplastic columnar epithelia in cancer patients.

Material and Methods

Tissue Samples

Tissue samples were obtained from patients with EAC, patients with BO, and controls (patients undergoing upper gastrointestinal endoscopy for dyspeptic symptoms but without endoscopic abnormalities) who presented to University Hospitals, Birmingham, UK, between May 2009 and March 2010. Overall ^{1}H-NMR spectra for 211 polar extracts from tissue samples were used in this study (Table 1).

Ethics Approval and Consent to Participate. Patients were recruited from University Hospitals Birmingham between May 2009 and March 2010. All patients included in this study gave informed consent. Ethical approval for this study was obtained from South Birmingham Research Ethics Committee (REC reference number 08/H1207/3).

Sample Collection. Healthy normal esophageal squamous mucosa biopsies were obtained from 68 patients presenting with symptoms of benign gastroesophageal reflux disease (NN). A total of 51 EAC patients contributed samples either pre- or postchemotherapy (or both). Their disease was staged as T2/3N0/1. Five EAC patients with other major comorbidities were not included in the subsequent analysis. There were 7 Barrett's patients who contributed both histologically normal and Barrett's tissue samples.

For patients with gastroesophageal malignancies, biopsies of tumor mucosa; histologically normal tissue at least 5 cm from tumor; and, if available, Barrett's mucosa were obtained under general anesthetic prior to staging laparoscopy. For some patients, a second set of samples was collected after chemotherapy. All diagnoses were histologically confirmed using biopsies. For Barrett's patients, biopsies were obtained at the time of endoscopy for Barrett's mucosa and for normal mucosa at least 5 cm from the Barrett's mucosa. Samples from controls were also obtained at the time of endoscopy. All tissue vials were stored on ice for 1 hour and then at −80°C. Patient and sample data are summarized in Table 1.

Sample Preparation. Methanol chloroform extraction, as originally described by Bligh and Dwyer [18], was used to prepare polar extracts for NMR analysis. Tissues were homogenized using a Precellys 24 ceramic bead-based homogenizer (Stretton Scientific Ltd., UK). All solvents were kept on ice. Eight microliters per milligram of methanol and 2.5 μl/mg of water were added to each Precellys tube, and tubes were placed in the Precellys 24 homogenizer for two 10-second bursts at 6400 rpm. The homogenized mixture was pipetted into a clean 1.8-ml glass vial using a Pasteur pipette. Eight microliters per milligram of chloroform and 4 μl/mg of water were subsequently added to each vial. The vials were vortexed at full power for 30 seconds each and left on ice for 10 minutes. They were then centrifuged at 1800*g* (3000 rpm) at 4°C for 10 minutes. The polar fraction was dried in a centrifugal evaporator (SpeedVac).

NMR Spectra

Data Acquisition. For NMR analysis, dried polar extracts were then resuspended in 100 mM sodium phosphate, pH 7, with 0.5 mM TSP as internal reference and 10% D2O as lock solvent. All ^{1}H Nuclear Overhauser Spectroscopy spectra were acquired on a 600-MHz Bruker AVANCE2 spectrometer with a 1.7-mm TXI probe at 288 K using the standard Bruker sequence, noesygppr1d with a very short Nuclear Overhauser Spectroscopy mixing time of 10 milliseconds and with a 9.8-microsecond 1H hard pulse at 17 dB. A total of 32 k points were acquired over an acquisition time of 2.2