Barrett’s Esophagus: Metaplastic Cells with Loss of Heterozygosity at the APC Gene Locus Are Clonal Precursors to Invasive Adenocarcinoma


Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20892; [Z. Z., A. O. V., M. R. E. B., M. J. M., L. A. L., P. H. D.]; Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts 02114 [E. J. M.]; Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts 02115 [R. O.]; and Department of Internal Medicine, Catholic University Medical College, Seoul, Korea [H. M.]

Abstract

Adenocarcinoma in Barrett’s esophagus is the second most rapidly increasing cancer in western society. The cause and pathogenesis are unknown. Although histological studies suggest that there is successive progression from metaplasia and dysplasia, with a high risk of subsequent invasive carcinoma, at present there is no direct evidence that metaplastic and dysplastic epithelia are clonal precursors of adenocarcinoma.

We selected 12 esophagectomy specimens of Barrett’s esophagus patients, which showed a spectrum of normal tissue, metaplasia, dysplasia, and invasive adenocarcinoma in each individual biopsy. We applied the microdissection technique to selectively procure microscopic tissue samples from H&E-stained slides for genetic evaluation using polymorphic markers flanking the APC gene locus.

Identical APC gene alterations were found in the dysplastic and adenocarcinoma foci of all informative cases. The same changes were observed even in some metaplastic foci adjacent to dysplasia. Furthermore, clonality analysis of X-chromosome inactivation in female cases verified the same X-chromosome inactivation pattern in carcinoma, dysplasia, and metaplasia adjacent to dysplasia. No APC gene alterations were found in the normal epithelium and metaplasia distant from dysplasia.

These data show for the first time that a tissue field of genotypic changes precedes the histopathological phenotypic changes of carcinoma in Barrett’s esophagus syndrome. Our findings, in conjunction with the applied tissue microdissection technique, may help identify genotypic changes in patients with Barrett’s esophagus before phenotypic changes occur. Therefore, genotyping of Barrett’s metaplastic epithelium may supplement the histopathological evaluation of Barrett’s esophagus.

Introduction

Barrett’s esophagus is associated with an increased risk of developing invasive adenocarcinoma. It is difficult to detect those patients at high risk for developing esophageal adenocarcinoma (1, 2), and most methods rely on the histological recognition of high-grade dysplasia from surveillance endoscopic biopsy specimens (3, 4). Histological dysplasia recognition is problematic in practice (5), and the frequency of endoscopic surveillance has been questioned (2, 6). Although patients with Barrett’s-associated adenocarcinoma show a spectrum of histological changes, such as metaplasia, dysplasia, and invasive adenocarcinoma, there is no direct evidence that metaplastic and dysplastic epithelia are clonal precursors of adenocarcinoma.

A recently developed tissue microdissection technique allows for selective procurement and genetic analysis of isolated, selected cell populations from paraffin-embedded, archival material (7-10). With this technique, genetic changes can be selectively investigated in different histological areas from a given tissue section. Therefore, this approach allows one to investigate whether specific genetic alterations are associated with a characteristic morphological phenotype and to analyze the spatial distribution of cellular genetic changes within their histological architectural context.

Recently, the APC gene has been mapped and sequenced. Alterations of this gene have been found in colon, stomach and esophageal tumors (11, 12). Studies on the evolution of colorectal cancer through the adenoma-carcinoma progression (11) have implicated APC gene alterations among the earliest genetic changes that occur during progression from a histologically benign to a malignant phenotype.

Therefore, the goal of this study was to investigate APC gene alterations in different histological regions representative of the potential stages of progression from BE2 to carcinoma. In addition, clonality studies were performed to assess whether BE, dysplasia and carcinoma are derived from the same cellular origin.

Materials and Methods

The pathological material of 20 recent surgical resections of Barrett’s esophagus performed at the Massachusetts General Hospital was reviewed. Twelve resection specimens were selected for the study, because normal epithelium, gastroesophageal junction, BE, dysplasia, and invasive carcinoma could be identified in one or two histological slides, unobserved by extensive inflammation or necrosis. From all 12 cases, at least three areas of invasive adenocarcinoma were microdissected directly from H&E-stained sections, as described earlier (7). The dissected tissue was placed in proteinase K buffer for DNA extraction. The DNA extraction mix was PCR amplified using the genetic polymorphic markers DSS299, DSS346, and DSS3I1 (Research Genetics, Huntsville, Alabama) for LOH of the APC gene locus. The amplified PCR products were analyzed on a denaturing 6% polyacrylamide gel, and subsequent autoradiography was used for visualization. The screening process of these 12 cases yielded 5 cases harboring deletion of the APC gene in the adenocarcinoma component and 5 cases with no deletion. Two cases were homozygous for all three polymorphic markers tested. Therefore, these cases were not informative and were not used for further genetic study.

In the five cases that showed APC gene deletions in the adenocarcinomatous component, the following areas of interest were additionally dissected (Fig. 1): (a) normal control tissue (esophageal squamous epithelium, gastroesophageal junctional epithelium, and lymphoid tissue); (b) BE distant from dysplasia (more than one ×10 power field = 2 mm separated from dysplastic area); (c) BE adjacent to dysplasia (less than one ×10 power field = 2 mm separated from dysplastic area); (d) dysplasia; and (e) invasive carcinoma.

Of the three possible histological subtypes of Barrett’s metaplastic epithelium (13), only the specialized “intestinal” type was targeted for this study. The criteria for recognition of epithelial dysplasia were those described previously (1, 3-5). In this study, no attempt was made to analyze high- and low-grade dysplasia separately. The dysplastic lesions selected for this study were exclusively from high-grade dysplasia.

All samples from the five informative cases were subjected to genetic analysis, as described above. X-chromosome inactivation clonality analysis

Received 1/29/96; accepted 3/18/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Laboratory of Pathology, National Cancer Institute, Building 10, Room 2N214, Bethesda, MD 20892.

2 The abbreviations used are: BE, Barrett’s epithelium; LOH, loss of heterozygosity.
was performed on two female cases using a polymorphic marker (Humara) on the X chromosome (14, 15).

Results

Five of 10 informative cases (heterozygous for the genetic polymorphic markers tested) of Barrett’s esophagus with dysplasia and invasive adenocarcinoma showed LOH of the APC gene in the adenocarcinoma. From these five cases, we analyzed different histological regions, including normal tissue, BE distant from dysplasia, BE adjacent to dysplasia, dysplasia, and adenocarcinoma.

Loss of the same allele at the APC gene locus was detected in both invasive adenocarcinoma and dysplasia in five of five cases (Fig. 2). A separate clonality analysis for X-chromosome inactivation in two female cases showed all lesions to be monoclonal. In each case, the clonal patterns of dysplasia and carcinoma were the same (Fig. 3).

BE adjacent to dysplasia showed LOH of the APC gene in two of five cases. The allelic loss was identical to that found in the adjacent dysplastic epithelium in both cases (Fig. 2). Furthermore, the female patient with LOH of the APC gene in BE adjacent to dysplasia showed monoclonality, and the clonal pattern was the same as those of the adjacent dysplasia and invasive adenocarcinoma (Fig. 3).

All five cases of BE distant from dysplasia and all normal tissues (esophageal squamous epithelium, gastroesophageal junctional epithelium, and lymphoid cells) showed no LOH of the APC gene (Fig. 2). The clonality analysis showed these lesions, as well as normal tissue, to be polyclonal (Fig. 3). Thus, the APC gene analysis, as well as the clonality assay, showed no difference between BE distant from dysplasia and normal control tissue.

Discussion

In the past few years, molecular studies of colorectal cancer have contributed to the identification of tumor-related genes in this cancer and also to the understanding of genes that are related to the development and progression of intestinal cancer (16). Both oncogenes and tumor suppressor genes play a role in tumorigenesis (17). The molecular abnormalities that have been reported in esophageal cancer include amplification of the proto-oncogenes MYC, INT2, HST1, and EGFR (18–21), overexpression, point mutations, and LOH of the p53
OKNKTIC CHANGES IN BARRETT'S ESOPHAGUS

gene (22–24) and tumor suppressor genes, including the APC and MCC genes (12).

Although the relative significance of the different gene alterations is not yet known, the study of these genes has provided firm evidence of the concept of multistep carcinogenesis (25). The APC gene has been implicated at an early stage of colorectal carcinogenesis (11), but the consequences of APC protein function in tumorigenesis are poorly understood (26). Although the regulatory roles for APC gene products await elucidation, it is clear that the APC gene and its molecular alterations are highly specific for gastrointestinal tumorigenesis.

The recently developed microdissection technique used in this study allows a powerful and cost-effective approach to screen for genetic changes in small and microscopically identifiable cell populations (7–10). It can be applied to paraffin-embedded, archival material and allows for selective analysis of cell populations with different phenotypes. In this study, we were able to selectively analyze genetic changes in metaplastic, dysplastic, and neoplastic epithelia in patients with Barrett’s esophagus and compare them with the patients’ constitutional genotypes by investigating normal esophageal mucosa or lymphoid tissue. This approach allowed us to identify the stage at which the APC gene alterations occurred in tumor development.

Similar to the colorectal carcinogenesis model (11), the BE model allows the study of genetic changes that occur during progression from a histologically benign to a malignant phenotype. However, in contrast to the colorectal carcinogenesis model, the BE model includes an early and focal metaplastic change, which subsequently progresses to cancer. This circumscribed metaplastic zone represents a pool of histologically normal-appearing, intestinal-type cells with an increased tendency toward malignant transformation. Thus, the BE model allows the study of the progression of a phenotypically normal epithelium to invasive adenocarcinoma (Fig. 4).

In this study, we found identical allelic loss patterns of the APC gene in BE, dysplasia, and invasive adenocarcinoma. Although we did not study the remaining APC gene allele, previous work has established that the remaining APC gene often shows mutations or small deletions in the presence of wild-type deletions during colorectal carcinogenesis (17, 27). Therefore, our results support the hypothesis that Barrett’s esophagus could be a precursor of dysplasia and subsequent invasive adenocarcinoma. Furthermore, X-chromosome inactivation clonality studies showed the same clonal pattern in metaplasia, dysplasia, and carcinoma, which suggests the emergence of a clonal population associated with LOH occurring in the metaplastic tissue adjacent to a dysplastic lesion, but not in the metaplastic epithelium distant from dysplasia. Thus, these data show that the genetically altered metaplastic epithelium represents a clonal precursor of adjacent carcinoma and strongly support the concept that a field of epithelial genotypic changes precedes the development of a malignant phenotype.

The results of this study may help predict the clinical course of patients with Barrett’s esophagus. Genotyping of BE may be an
important part of a complete assessment of patients with this disease and may carry significant information. Early identification of genotypic changes in Barrett’s esophagus, before the appearance of dysplasia or adenocarcinoma, may provide information for assessment, monitoring, and therapeutic strategies of individual patients.

References


Barrett's Esophagus: Metaplastic Cells with Loss of Heterozygosity at the APC Gene Locus Are Clonal Precursors to Invasive Adenocarcinoma


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/56/9/1961

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.