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Original contribution

Fluorescence in situ hybridization mapping of esophagectomy specimens from patients with Barrett's esophagus with high-grade dysplasia or adenocarcinoma

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Summary The progression of intestinal metaplasia to esophageal adenocarcinoma in patients with Barrett's esophagus is partly driven by chromosomal alterations that activate oncogenes and inactivate tumor suppressor genes. The goal of this study was to determine how alterations of 4 frequently affected genes correlate with the range of histopathologic lesions observed in resected esophagi of patients with Barrett's esophagus. Fluorescence in situ hybridization was used to assess 83 tissue sections from 10 Barrett's esophagus esophagogastrectomy specimens for chromosomal alterations of 8q24 (*MYC*), 9p21 (*CDKN2A*; alias *P16*), 17q12 (*ERBB2*), and 20q13.2 (*ZNF217*). Histologic lesions assessed included gastric metaplasia (n = 8), intestinal metaplasia (n = 43), low-grade dysplasia (n = 28), high-grade dysplasia (n = 25), and adenocarcinoma (n = 16). Histologic maps showing the correlation between fluorescence in situ hybridization abnormalities and corresponding histology were created for all patients. Chromosomal abnormalities included 9p21 loss, single locus gain, and polysomy. A greater number of chromosomal alterations were detected as the severity of histologic diagnosis increased from intestinal metaplasia to adenocarcinoma. All patients had alterations involving the *CDKN2A* gene. *CDKN2A* loss was the only abnormality detected in 20 (47%) of 43 areas of intestinal metaplasia. Polysomy, the most common abnormality in dysplastic epithelium and adenocarcinoma, was observed in 16 (57%) of 28 low-grade dysplasia, 22 (88%) of 25 high-grade dysplasia, and 16 (100%) of 16 adenocarcinoma. The findings of this study improve our understanding of the role that chromosomal instability and alterations of tumor suppressor genes such as *CDKN2A* and oncogenes such as *ERBB2* play in the progression of intestinal metaplasia to adenocarcinoma in patients with Barrett's esophagus.

1. Introduction

The incidence of esophageal adenocarcinoma (EA) is on the rise in the United States, increasing more rapidly than the rate of melanoma and breast and prostate cancer [1]. Barrett's

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esophagus (BE), a change in the distal esophageal epithelium from stratified squamous epithelium to columnar type mucosa, is the only known precursor of EA. The progression of intestinal metaplasia (IM) to EA occurs through a multistep dysplasia carcinoma sequence [2].

In the 1980s, Fearon et al [3] demonstrated that colorectal tumor progression from normal to adenoma to carcinoma was associated with the accumulation of mutations in oncogenes (eg, *KRAS*) and tumor suppressor genes (eg, *TP53*). They also demonstrated that carcinoma had more genetic alterations than large adenomas, which, in turn, had more than small adenomas. They proposed that the accumulation of mutations in oncogenes and tumor suppressor genes drove tumor progression. Since that time, other investigators have demonstrated similar findings for a number of different tumor types such as lung, breast, and pancreatic cancer [4-6].

Studies of patients with BE-associated neoplasia have shown that the progression of normal squamous epithelium to EA in patients with BE results from the accumulation of genetic or epigenetic alterations in tumor suppressor genes (eg, *CDKN2A* and *TP53*) and oncogenes (eg, *ERBB2*) [7-9]. Alterations in these genes lead to malignant phenotypic characteristics such as uncontrolled proliferation, resistance to apoptosis, or invasiveness. These acquired properties can lead to clonal expansion and increased tumor aggressiveness [10].

Fluorescence in situ hybridization (FISH) is a technique that can be used to detect gene copy number abnormalities (eg, aneuploidy, gene deletion, and amplification) in cells. We have recently developed a FISH probe set containing probes for 8q24 (*MYC*), 9p21 (*CDKN2A*), 17q12 (*ERBB2*), and 20q13.2 (*ZNF217*) to detect chromosomal alterations that are involved in the progression of IM to EA in patients with BE [11,12].

In the late 1990s, investigators from our institution carefully mapped the histologic lesions present in esophagectomy specimens from patients with BE who had an esophagectomy because of a high-grade dysplasia (HGD) or EA biopsy diagnosis [13]. This was the largest study of its type and showed the range and distribution of histologic lesions present throughout the esophagi from such patients. The goal of the current study was to use the specimens from the previous mapping study to determine how chromosomal alterations implicated in BE-associated neoplasia correlate with morphological changes and tumor progression.

2. Materials and methods

2.1. Patient population

Esophagogastrectomy specimens were obtained from 10 patients with BE (7 men and 3 women) who had undergone esophageal resection between January 1992 and December 1994 at Mayo Clinic, Rochester. The patients' median age was 65 years, with a range of 36 to 75 years. Of the 10 patients, 7 had either a preoperative endoscopic biopsy or postoperative histologic diagnosis of EA, whereas the remaining 3 patients were found to have HGD.

2.2. Specimen selection

Eighty-three formalin-fixed, paraffin-embedded tissue blocks (range, 8-10 blocks per patient) were selected from the 10 esophagi for FISH analysis after approval by the Mayo Clinic Institutional Review Board. Previously constructed histologic maps of the resected esophagi [13] were used to select tissue blocks that exhibited varying histopathologic diagnoses. Two 5-μm slides were cut from each of the 83 blocks. One slide was prepared for FISH analysis, and 1 was stained with hematoxylin and eosin. The hematoxylin and eosin slides were reviewed by a pathologist (K. C. H.), and 130 areas (9-19 areas per patient) were identified and marked for FISH analysis. Selected areas represented the various histologic categories present in each patient's esophagus. An area of squamous epithelium from each patient was also selected for analysis.

2.3. Histopathology review

To provide correlation between chromosomal status and histologic classification, areas used for FISH analysis were reviewed by 2 trained pathologists (T. C. S. and K. C. H.). Histologic diagnoses were classified as gastric metaplasia, IM, low-grade dysplasia (LGD), HGD, or EA.

2.4. FISH processing

Slides were processed for FISH analysis as previously described [14], with the following exceptions: (1) during deparaffinization, slides were heated to 75°C rather than 90°C; (2) a 70% alcohol solution was used for dehydration instead of a 75% alcohol solution; (3) a multicolor FISH probe set containing probes for the 8q24 (*MYC*), 9p21 (*CDKN2A*), 17q12 (*ERBB2*), and 20q13.2 (*ZNF217*) loci (Abbott Molecular Inc, Des Plaines, IL) was used.

2.5. FISH analysis

FISH analysis was performed by an experienced technologist without knowledge of the corresponding histologic diagnosis. The nuclear counterstain and probe signals were viewed using a fluorescence microscope equipped with filters specific for 4,6-diamidino-2-phenylindole, Spectrum Aqua, Spectrum Green, Spectrum Gold, and Spectrum Red (Abbott Molecular Inc, Des Plaines, IL). Within designated areas of squamous epithelium, 100 consecutive cells were enumerated; and signal patterns were recorded. In the nonsquamous