A case of early Barrett’s adenocarcinoma repeatedly developing multiple metachronous lesions shortly after endoscopic therapy: an analysis for genetic and epigenetic alterations

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-Case report-

A case of early Barrett’s adenocarcinoma repeatedly developing multiple metachronous lesions in a short period following endoscopic therapy: an analysis for genetic and epigenetic alterations

A brief title: Barrett’s adenocarcinoma repeating metachronous lesions

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Introduction

Barrett’s esophagus (BE) is a premalignant condition and the most important known risk factor for esophageal adenocarcinoma (EAC) [1]. Although intestinal metaplasia (IM) in BE mucosa is considered a precursor for EAC, there is a lack of universal agreement regarding the inclusion of IM as a criterion for the diagnosis of BE between the US and Europe [2,3]. We report herein a case that repeatedly developed multiple metachronous cancers at the BE mucosa without IM after undergoing endoscopic treatment for early EAC. Additionally, we evaluated the chronology of genetic and epigenetic events as well as cellular phenotype, as identified by a monoclonal antibody (mAb) Das-1 related to carcinogenesis that specifically (100%) reacts with BE and EAC [4,5].

CASE REPORT

A 59-year-old man underwent upper gastrointestinal endoscopy as part of his routine medical check-up in April 2009, at which time an elevated lesion in the esophagus was detected. He had a history of polycystic kidney, and he had received regular dialysis treatment, but did not have a family history of malignancies. Upper endoscopy revealed an elevated lesion 1 cm in diameter in a long-segment BE approximately 12 cm long. The lesion showed a sharply marginated and irregular surface on chromoendoscopy (Fig. 1). Magnified high-resolution narrow band imaging (NBI) revealed that the microvessels of the tumor surface were irregular, and the diameters and distribution of the vessels were heterogeneous. As early EAC was suspected on the endoscopic findings including NBI and biopsies, cap-assisted endoscopic mucosal resection (c-EMR) was performed after obtaining written informed consent. The resected area was 26 x 21 mm, and complete en bloc resection with
tumor-free margins was accomplished histopathologically. The histology revealed intramucosal moderately differentiated adenocarcinoma with lymphatic invasion in the muscularis mucosae, and did not demonstrate any findings of IM with goblet cells and dysplastic glands surrounding the malignancy. Follow-up esophagoscopy at 2 months after the initial EMR identified multiple areas of irregular mucosa around the EMR scar, and the biopsy samples were diagnosed histologically as well-differentiated adenocarcinoma (Fig. 2). Thereafter, extensive contact thermal ablation (CTA) with c-EMR was performed on the lesions. The CTA procedure was performed with the lateral of hot biopsy forceps (FD-1L-1, Olympus, Tokyo) with a 40W soft-mode coagulation current (effect 5) with an electrosurgical unit (VIO 300D, ERBE, Germany). Two months later, following additional endoscopic therapy, cancers developed again around the scar (Fig. 3). Finally, the remaining BE was completely eradicated with 7 sessions of EMR and/or extensive ablation therapy within 10 months.

We evaluated the microsatellite instability (MSI), the methylation status, and immunoreactivity using a mAb Das-1 in the cancer and the background mucosa according to our previous reports [4-7]. Both cancer and biopsy materials from the BE mucosa were found to be positive for MSI at the locus of D5S346 and D17S250 among 5 loci investigated [8]. The promoter methylation at the APC gene was present under the two conditions (Fig. 4). However, mAb Das-1 reacted with the cancer area but not with the BE mucosa.

**DISCUSSION**

Recent publications by some investigators and groups have reported on short- to long-term data in patients with high-grade intraepithelial neoplasia and early cancer treated by various modalities of endoscopic therapy. During follow-up examinations, metachronous
neoplasia have been shown to the major problem with endoscopic therapy [9-13]. In the current case, all lesions developed around the scar after endoscopic therapy. Although the reasons why the multiple metachronous lesions became visible adjacent to the treated region in the short term remain unclear, there are a few explanations for the phenomenon. First, it was possible that these lesions were missed at the initial and follow-up endoscopy. However those lesions were not detected even though BE mucosa was observed thoroughly by chromoendoscopy and NBI prior to the initial treatment for EAC. Second, occult cancers that are not detectable at the time of endoscopic therapy may grow within a short period in response to a stimulus such as a growth factor [14,15] during the healing process of the endoscopic treated area. However, no evidence was found of dysplastic glands and IM in 24 biopsy specimens from long-segment BE, including the surrounding mucosa of the tumor. Pech et al. have described that the majority of recurrences or metachronous neoplasia found during follow-up are de novo neoplasias, and that these lesions usually develop due to genetic abnormalities within the remaining BE mucosa and are not influenced by endoscopic therapy [10].

Recently, Liu et al. have shown that DNA content abnormalities occur with equal frequency and extent in metaplastic columnar epithelium of the esophagus without IM compared with metaplastic columnar epithelium with IM [16]. We have previously reported genetic instability and epigenetic abnormalities, including *APC*, in BE with/without IM [6,7]. Furthermore, it has recently been reported that hypermethylation of *APC* strongly predict the progression to cancer in patients with BE [17]. These results indicate that EAC patients with genetic instability and *APC* hypermethylation in the background mucosa may be at a high risk of metachronous lesions. It has been reported that mAb Das-1 may allow early detection of
metaplastic epithelium of the colonic phenotype in the absence of histological evidence of specialized columnar epithelium [5]. In our case, however, columnar-lined epithelium showed negative staining to this antibody.

Taking previous reports [16,17] and the current case together, EAC patients with genetic instability and epigenetic alterations in the background BE mucosa may be at risk for recurrence even if IM is not observed in the biopsy samples. Thus, BE with those DNA alterations should be completely eradicated and be followed-up more intensively after endoscopic treatment.
References


Figure legends

Figure 1. A, Chromoendoscopy showed a sharply marginated elevated Barrett’s adenocarcinoma. B, With Magnified high-resolution NBI, irregular vascular pattern with abnormal blood vessels was found. C, The resected specimen revealed intramucosal moderately differentiated adenocarcinoma with lymphatic invasion in the muscularis mucosae.

Figure 2. A, Two months after the initial endoscopic mucosal resection (EMR), multiple areas of irregular mucosa (arrows) around the EMR scar (asterisk) was identified. The biopsy samples obtained from irregular mucosa revealed well-differentiated to papillary adenocarcinoma. B, The EMR specimens showed intramucosal papillary adenocarcinoma.

Figure 3. A, Follow-up esophagoscopy with NBI at 2 months after the second EMR identified multiple brownish irregular mucosa (arrowheads) around the endoscopic treatment (asterisk). B, The biopsy samples confirmed well-differentiated adenocarcinoma.

Figure 4. A, Microsatellite instabilities on D5S346 and D17S250 were seen as an unequivocal extra peak shift (asterisks) in both Barrett’s esophagus (BE) and esophageal adenocarcinoma (EAC) compared with normal gastric mucosa (control). B, The promoter methylation of APC was present in both BE and EAC (arrows).
Figure 4