

Case report: esophageal collision tumor (oat cell carcinoma and adenocarcinoma) in Barrett's esophagus: immunohistochemical, electron microscopy and LOH analysis

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Summary: We report a case of an esophageal collision tumor composed of adenocarcinoma and oat cell carcinoma. Both tumors appeared to arise from dysplastic Barrett's mucosae in a 75-year-old man. Immunohistochemical stains and electron microscopy demonstrated a separate identity for each of the tumors in collision. Molecular analysis of microsatellite regions was performed in different microdissected areas. Identical loss of heterozygosity (LOH) at 9p21 and 17p13 was determined in the three different microdissected areas of the adenocarcinoma component. LOH was not determined in any area of the oat cell carcinoma. This is the first study that analyzes the allele status of an esophageal collision tumor. Our findings suggest a biclonal origin for both components of the collision tumor.

Key words. Oat cell carcinoma, Collision tumor, Barrett's esophagus, Immunohistochemistry, Electron microscopy, LOH

Introduction

Adenocarcinoma is the most frequent malignant tumor that arises from Barrett's esophagus (Rosai, 1980). Less common malignant neoplasms in this location include leiomyosarcomas, gastrointestinal stromal tumors, carcinoid tumors, small cell carcinomas, carcinosarcomas and lymphomas (Levis and Appelman, 1995). The criterion for a collision tumor is the concrescence of two neighboring independent neoplasms that expand into each other (Dodge, 1961). An esophageal collision tumor composed of adenocarcinoma and neuroendocrine carcinoma is a very

infrequent event. On review of the literature, we found eight reported cases of collision tumor in the lower esophagus and gastroesophageal junction. The collision of a small cell carcinoma with an adenocarcinoma was reported in four cases (Dodge et al., 1961; Purdy and Gaffney, 1986; Saw et al., 1997). The collision of a large cell carcinoma with an adenocarcinoma was reported in one case (Wilson et al., 2000). A squamous cell carcinoma combined with an adenocarcinoma was found in the other three cases (Majmudar et al., 1978; Spagnolo and Heenan, 1980). We report the ninth case of a collision tumor composed of an adenocarcinoma and a neuroendocrine carcinoma.

Materials and methods

Case report

A 75-year-old man presented with a 4-year history of gastroesophageal reflux and Barrett's metaplasia without dysplasia of the esophagus. He had anorexia, weight loss and dysphagia. Clinical workup via endoscopic studies showed a polypoid mass at the gastroesophageal junction. Histological examination of the biopsy specimens established a preoperative diagnosis of collision tumor composed of oat cell carcinoma and adenocarcinoma. The patient was treated by thoracotomy and partial esophagogastrectomy and is presently undergoing chemotherapy.

Immunohistochemistry

Tissue samples were routinely fixed in 10% formalin, embedded in paraffin and cut at 5 μ m. Standard peroxidase-antiperoxidase techniques were performed with the DAKO ENVISION II kit (DAKO DIAGNOSTICOS, Barcelona). The following standard primary antibodies were used: cytokeratins AE1/AE3; S100 protein; chromogranin; neuron-specific enolase

(NSE); synaptophysin; HMB45; Leu7; CEA; CD45; ACTH; glucagon; insulin; somatostatin and gastrin.

Electron microscopy

Multiple diced 1 mm slices from the formalin-fixed resection specimen were washed in buffer, placed in 3% glutaraldehyde and post-fixed in osmium tetroxide. The tissue was processed routinely and embedded in araldite. Ultrathin sections of selected blocks were examined in a Jeol 100 cx transmission electron microscope.

Molecular analysis

Different cell populations were microdissected with a disposable, modified, 30-gauge needle from paraffin-embedded tissues. Microdissected procured cells were resuspended in a solution containing 0.1 mg/ml proteinase K, and incubated 48 hours at 55 °C. The mixture was boiled for 10 minutes to inactivate proteinase K. All the cell populations were subjected to PCR analysis. Oligonucleotide primers flanking microsatellite polymorphisms at 5q21 (346), 3p21 (D3S1300), 9p21 (D9S157) and 17p13 (D17S799) were used in the study. Reactions were cycled as follows: 94 °C for 1min, 55 °C for 1 min, 72 °C for 1 min, with a total of 35 cycles. 1 ul of labeled amplified DNA was mixed with 12.5 μ l of formamide and 0.5 μ l of Genescan 500 TAMRA. The samples were denatured for 5 min at

95 °C and loaded into the Perkin-Elmer ABIPRISM 310 analyzer. Lymphocytes were considered to be representative of the normal DNA of the patient

Results

Gross pathology

The surgical specimen consisted of 19 cm of the distal esophagus and 9 of the stomach. An elevated brittle mass, measuring 4.5x4 cm, was determined at the gastroesophageal junction involving 80% of the esophageal circumference and the proximal portion of the stomach. A total of 13 paraesophageal and perigastric lymph nodes were dissected.

Light microscopy

Microscopic examination revealed Barrett's metaplasia and severe dysplasia (intraepithelial neoplasia). Two histologically distinct neoplastic growths had arisen from the severe dysplasia area (Fig. 1). One of them consisted of an oat cell carcinoma showing a diffuse pattern of growth. Solid sheets, ribbons and nests of tumor cells infiltrated all the muscular propria thickness but not the subserosa layer. Tumor cells exhibited scanty cytoplasm with intermediate-sized nuclei and frequent mitotic figures. The other tumor consisted of a well-differentiated adenocarcinoma with occasional signet-ring cells,

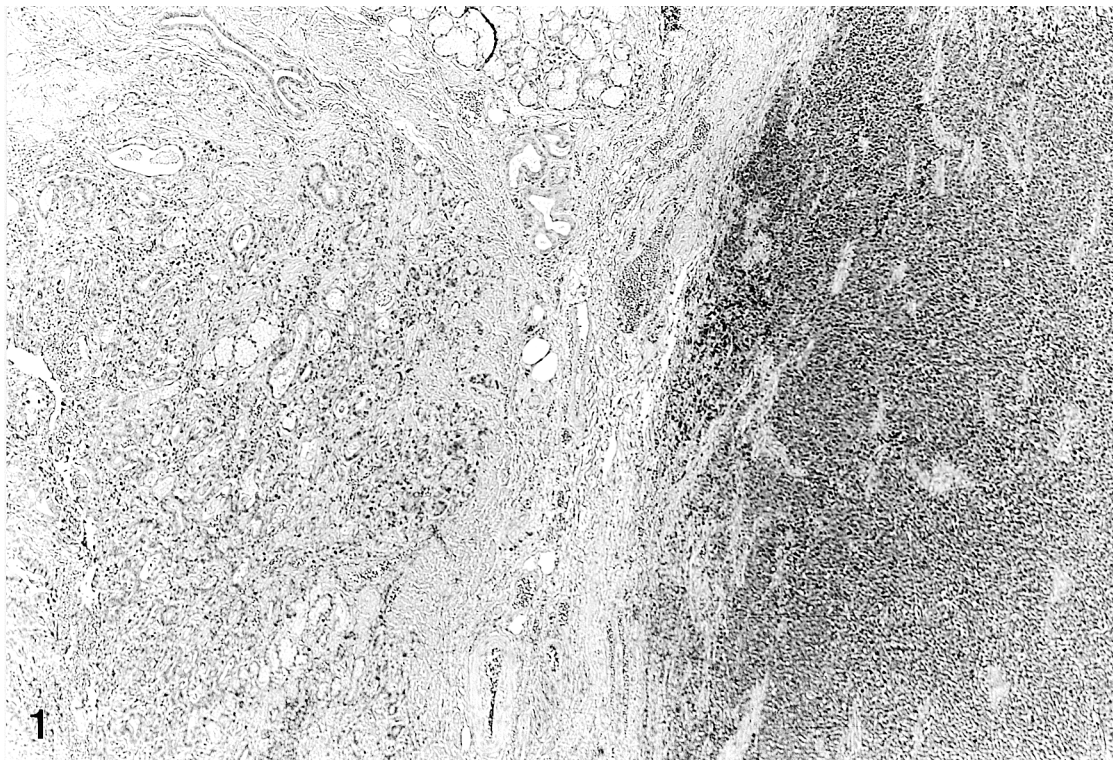


Fig. 1. Esophageal collision tumor: On the right side the oat cell carcinoma and on the left the adenocarcinoma. (H-E, x 4).

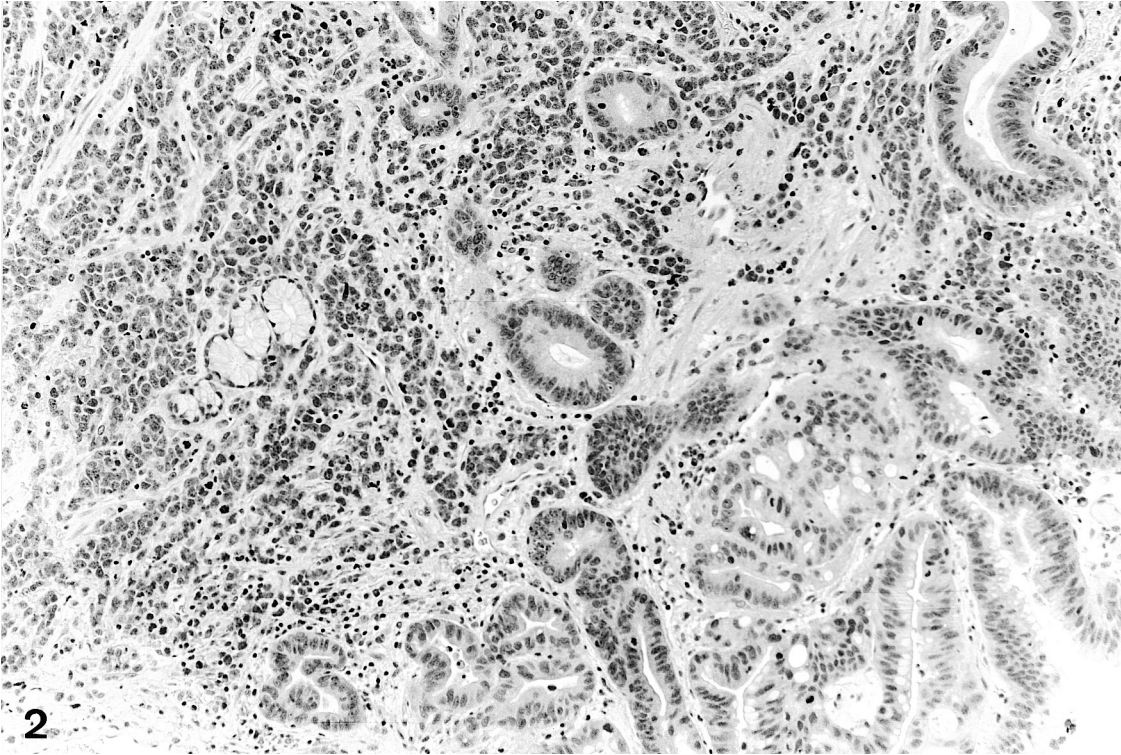


Fig. 2. Minimal intermingling of well-differentiated adenocarcinoma and oat cell carcinoma. H-E, x 20

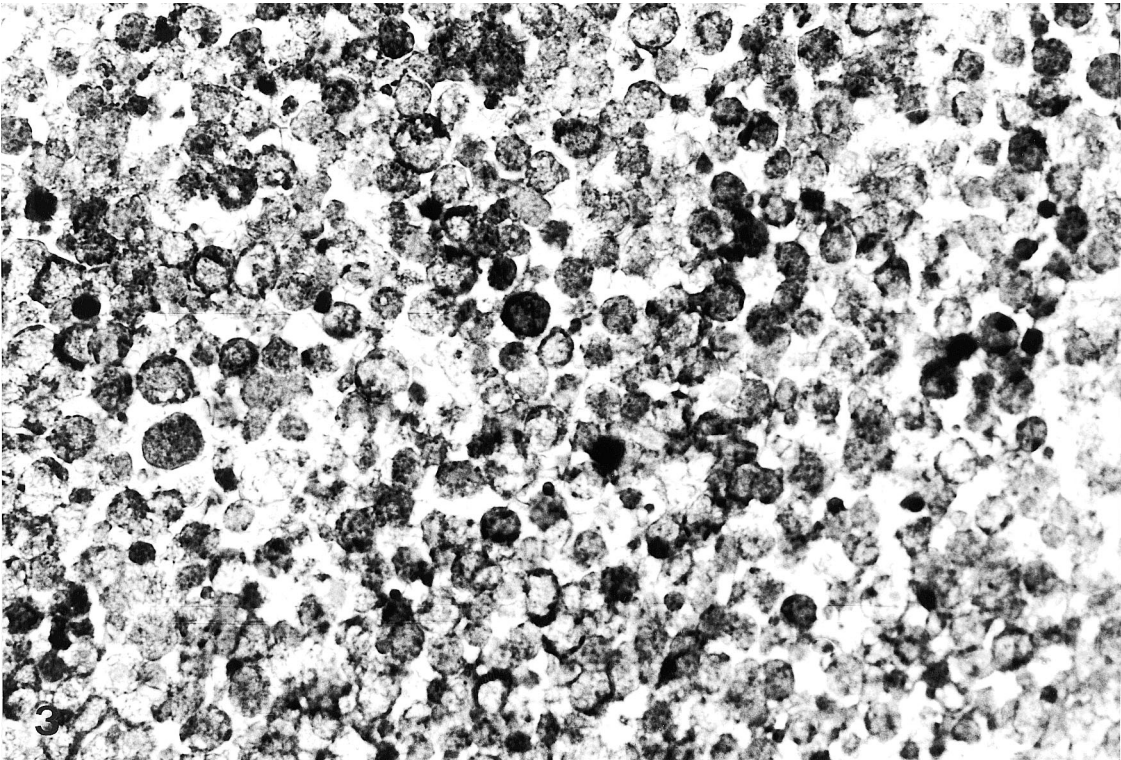


Fig. 3. Strong and diffuse positivity for Neuronal Specific Enolase. NSE, x 40

infiltrating only submucosa. Minimal intermingling of the two tumors was detected (Fig. 2). No lymph node metastasis was detected in any of the 13 dissected lymph nodes (0/13).

Immunohistochemistry

Oat cell undifferentiated carcinoma showed strong and diffuse positivity for LEU-7 and NSE (Fig. 3) with focal and slight reactivity for synaptophysin. Adenocarcinoma cells showed reactivity for CEA. Cytokeratins, chromogranin, S100, CD45, HMB45, gastrin, somatostatin, glucagon, insulin and ACTH immunostaining were negative in both tumors.

Electron microscopy

The ultrastructural examination showed small electron dense granules in the cytoplasm and desmosomal intercellular junctions in the oat cell carcinoma component (Fig. 4).

Molecular analysis

Different cell populations were microdissected from

Table 1. LOH analyses.

LOCUS	AC-1	AC-2	AC-3	OC-1	OC-2	NORMAL
5q21	Het	Het	Het	Het	Het	Het
3p21	Homo	Homo	Homo	Homo	Homo	Homo
9p21	LOH	LOH	LOH	Het	Het	Het
17p13	LOH	LOH	LOH	Het	Het	Het

AC-1, 2 and 3: three different microdissected areas of adenocarcinoma; OC-1 and 2: two microdissected areas of oat cell carcinoma; Het: Heterozygous; LOH: loss of heterozygosity; Homo: Homozygous.

paraffin-embedded tissues: 2 oat cell carcinoma areas, 3 adenocarcinoma areas and normal lymphocytes.

The analysis demonstrated that the patient was heterozygous at 5q21, 9p21 and 17p13 and homozygous at 3p21. The criterion for LOH was reduction in the lesional DNA allele by at least 70%. LOH was determined at 9p21 and 17p13 in 3 out of 3 oat cell carcinoma areas and was not detected in adenocarcinoma (Table 1).

Discussion

The distinction between a collision tumor and a multi-directional differentiation of the same neoplasm may be difficult (Sasajima et al., 1988). Although usually considered to be merely an academic curiosity, collision tumors are clinically relevant in that the individual tumors may require different treatments. Molecular data may complement the morphological criteria to determine the biclonal origin of a collision tumor. There are very few reports in the literature where different somatic genetic alterations have been determined in the two components of a collision tumor (Fujii et al., 2000; Pavelic et al., 2000 Kersemaekers et al., 2000).

LOH at 3p21, 5q21, 9p21 and 17p13 has been reported to be an early event in carcinogenesis (Gonzalez and Artinez, 1997). Identical LOH at 5q21 has been determined in both adenocarcinoma cells and peritumoral Barrett metaplasia of the esophagus, suggesting that LOH precedes malignization (Zhuang and Vortmeyer, 1996). The present case is the first one to analyze the allele status of an esophageal collision tumor. Electron microscopy features, immunohistochemical stains and molecular analysis represent a further differentiation of the two components of the collision tumor. Indeed, the different genotypic changes

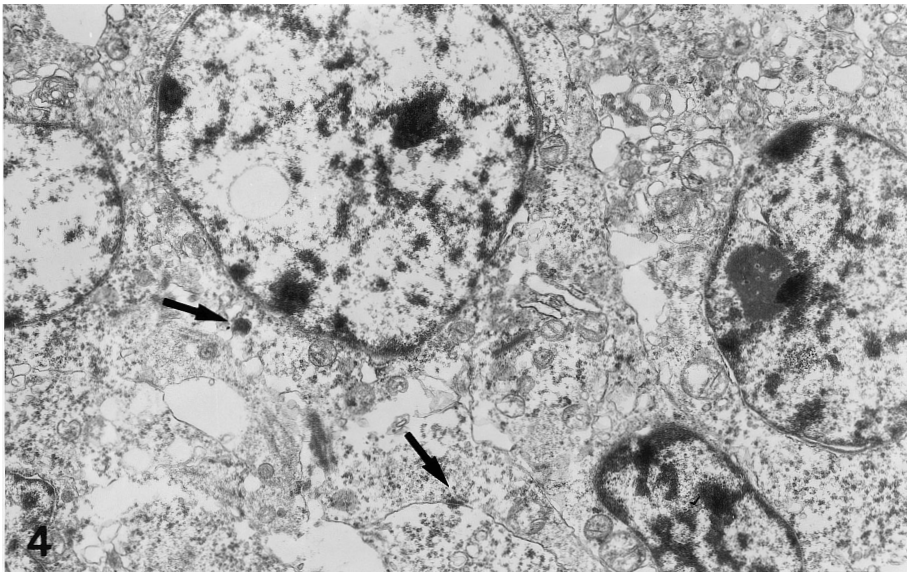


Fig. 4. Ultrastructural findings of oat cell carcinoma component: Electron-dense granules in the cytoplasm (top arrow) and desmosomal intercellular junctions (lower arrow). x 5,000

Esophageal collision tumor

we found are consistent with the hypothesis of a biclonal histogenesis for both components of the reported collision tumor.

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