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CASE REPORT

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Pyloric gland adenoma arising in Barrett's esophagus with mucin immunohistochemical and molecular cytogenetic evaluation

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Abstract Pyloric gland adenoma is a recently described and very rare entity. The occurrence of adenoma is very unusual in Barrett's epithelium of the esophagus. We report a case of esophageal polyp showing the features of pyloric gland adenoma, which was surrounded by so-called specialized columnar epithelium. Immunohistochemically, most tumor glands were strongly positive for MUC6, except in the superficial layer. MUC5AC was positive in almost all tumor cells, but MUC2 and CD10 were negative in the tumor. MIB-1-positive proliferating cells were distributed throughout the tumor. Microdissection and comparative genomic hybridization analyses revealed losses on 2p24–25.2, 2q14.1-ter, 5q31.3–32, 6q23–24, 8q23–24.2, 11q22.3–24 and 18q21.1–22. This is the first case of pyloric gland adenoma found to arise in Barrett's epithelium of the esophagus, showing its unstable and precancerous nature.

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Introduction

In Barrett's epithelium (BE), a rising incidence of adenocarcinoma has been described in the literature over recent decades. These adenocarcinomas are believed to develop through a metaplasia-dysplasia-carcinoma sequence [5, 6]. Intraepithelial neoplasia (dysplasia) may be broadly categorized as flat or associated with visible or raised lesions similar to those occurring in inflammatory bowel disease [20]. However, the occurrence of adenoma, a polypoid lesion composed of densely packed neoplastic glands similar to a colonic adenoma, is very unusual, and some authors have recently proposed a descriptive diagnostic term, such as BE-associated polypoid dysplasia [18].

Pyloric gland adenoma was first characterized by Borchard et al. [2], and Watanabe [26] described a similar lesion in the World Health Organization classification of gastric tumors in 1990. Borchard presented two cases of pyloric gland adenoma with transition to adenocarcinoma during two slide seminars in 1986 and 1987 (F. Borchard, personal communication). Similar adenomas of the gastric type were subsequently described in gastric heterotopia in the duodenum [14], in the pancreatic duct [1] and, in particular, in the gallbladder [7, 11, 17]. We recently reported a clinico-pathological analysis of 90 cases of pyloric gland adenoma [24].

The present study documents the first case of pyloric gland adenoma arising in BE. The cellular differentiation and molecular cytogenetic changes in this peculiar tumor are evaluated by means of mucin immunohistochemistry and comparative genomic hybridization (CGH).

Clinical summary

A 62-year-old Caucasian man had been followed up because of long-segment BE. In 2002, an upper gastrointes-

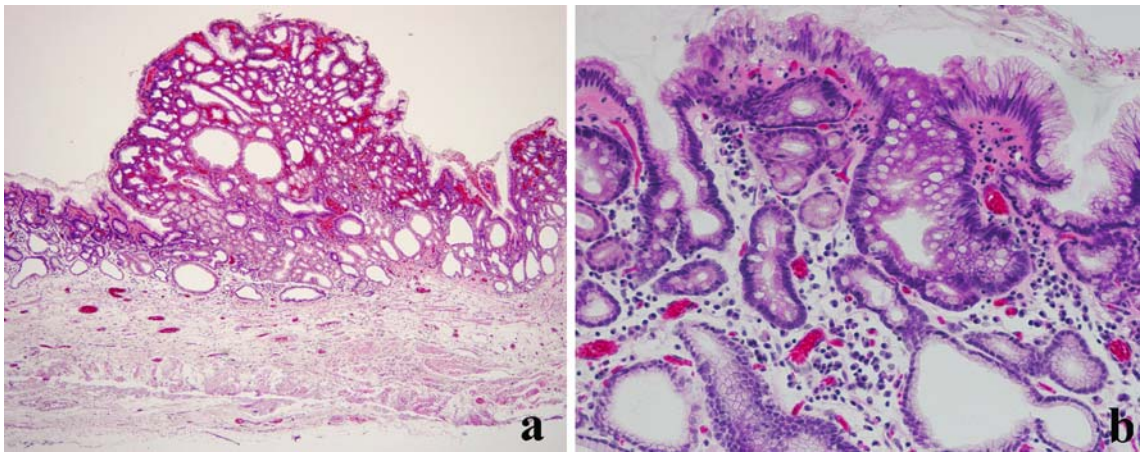

Fig. 1 a "Pyloric gland adenoma" surrounded by specialized columnar epithelium (SCE). Two-layered muscularis mucosae are seen. **b** Higher magnification of the SCE

tinal endoscopy disclosed a small nodular and polypoid lesion in BE, 25 cm distant from the incisors. Because of the suspicion that the tumor might be an early BE adenocarcinoma, the patient underwent an endoscopic resection of the questionable lesion. The lesion was completely removed with clear vertical and lateral margins. The patient is well after 3 years follow-up and developed no further neoplasia within his Barrett's segment.

Materials and methods

The tumor measured 3×2 mm with surrounding esophageal mucosa, was fixed in 10% formalin, sliced into two sections and embedded in paraffin. Paraffin blocks were available for routine histology and immunohistochemical studies. Immunohistochemical staining was carried out with the following monoclonal antibodies (mAbs): (1) MUC2 (Ccp58, 1:100; Novocastra, UK); (2) MUC5AC (CLH2, 1:100; Novocastra, UK); (3) MUC6 (CLH5, 1:100; Novocastra, UK) and (4) CD10 (56C6, 1:50; Novocastra, UK). A series of MUC2, MUC5AC, MUC6 and CD10 stains has been reported to be very useful in detecting the phenotypic expression of epithelial lesions of the gastrointestinal tract [16, 21]. MUC5AC, MUC6 and MUC2 specifically express, in gastric surface, mucous cells, pyloric gland cells and intestinal goblet cells of the mature gastrointestinal tract, respectively. MAb CD10 was used to detect the brush border of small intestinal absorptive cells and mAb MIB-1 to estimate the cell proliferation. The streptavidin-biotin method of detection was used. Peroxidase binding sites were visualized using the diaminobenzidine method, and the sections were lightly counterstained with hematoxylin.

We carried out microdissection of tumor cells from one 5-μm tissue section as described previously elsewhere [19]. Subsequently, these microdissected tissues were incubated in the proteinase K digestion. From the sample DNAs, whole genome was amplified using the method of degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) [9]. We used 1 μl of DOP-PCR product as tumor DNA and gender-matched reference DNA and labeled with Cy 3-dCTP and Cy 5-dCTP (Perkin Elmer,

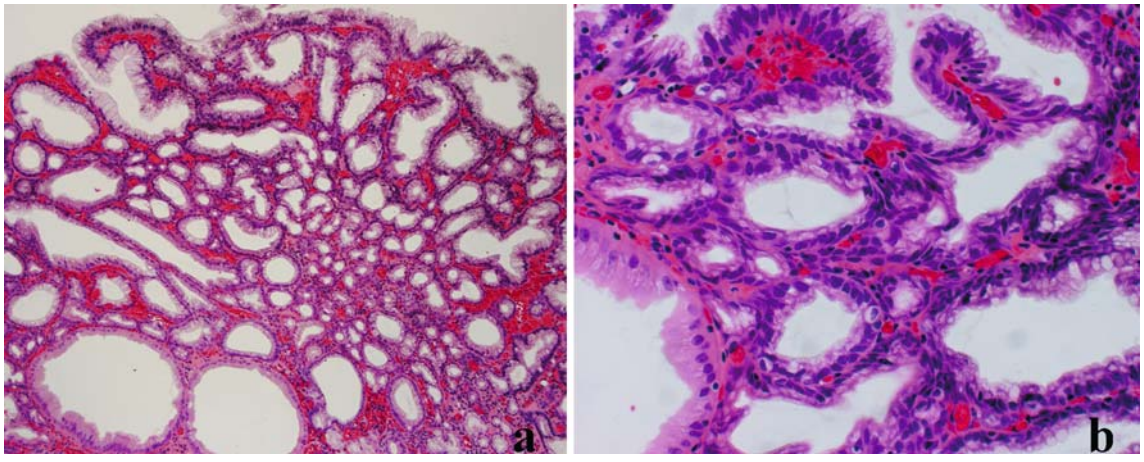

Fig. 2 Histological demonstration of the tumor. **a** The tumor consists of narrow to dilated tubules. They are lined by cuboidal to prismatic columnar epithelial cells that contain clear or pale eosinophilic cytoplasm. Superficial tumor cells were slightly higher in size than deeper ones. **b** Higher magnification of **a**. The tumor cell nuclei are small and oval or round in shape and contain small but conspicuous nucleoli

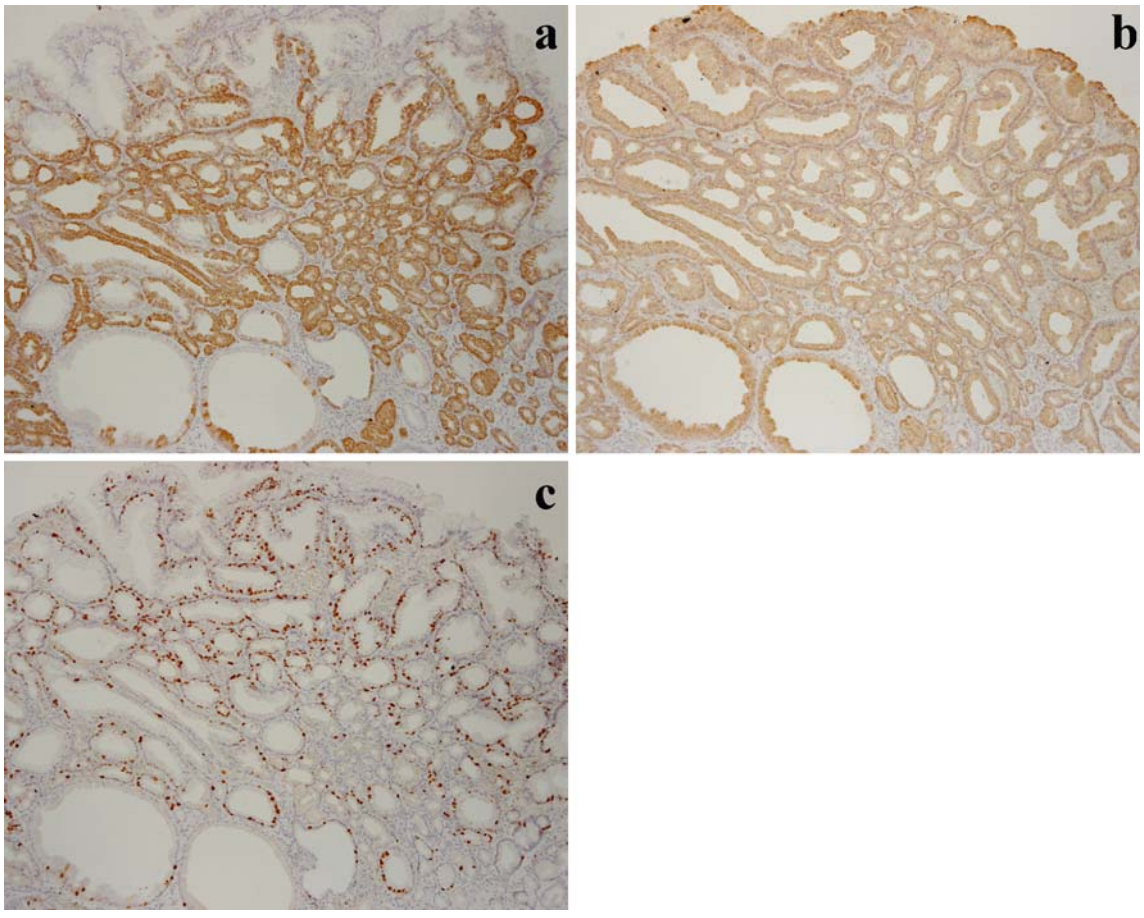

Fig. 3 Immunohistochemical demonstration of the tumor (serial sections of Fig. 2). **a** Most tumor glands are strongly positive for MUC6, except in the superficial layer. **b** MUC5AC is positive in almost all tumor cells. **c** MIB-1-positive proliferating cells are distributed throughout the tumor

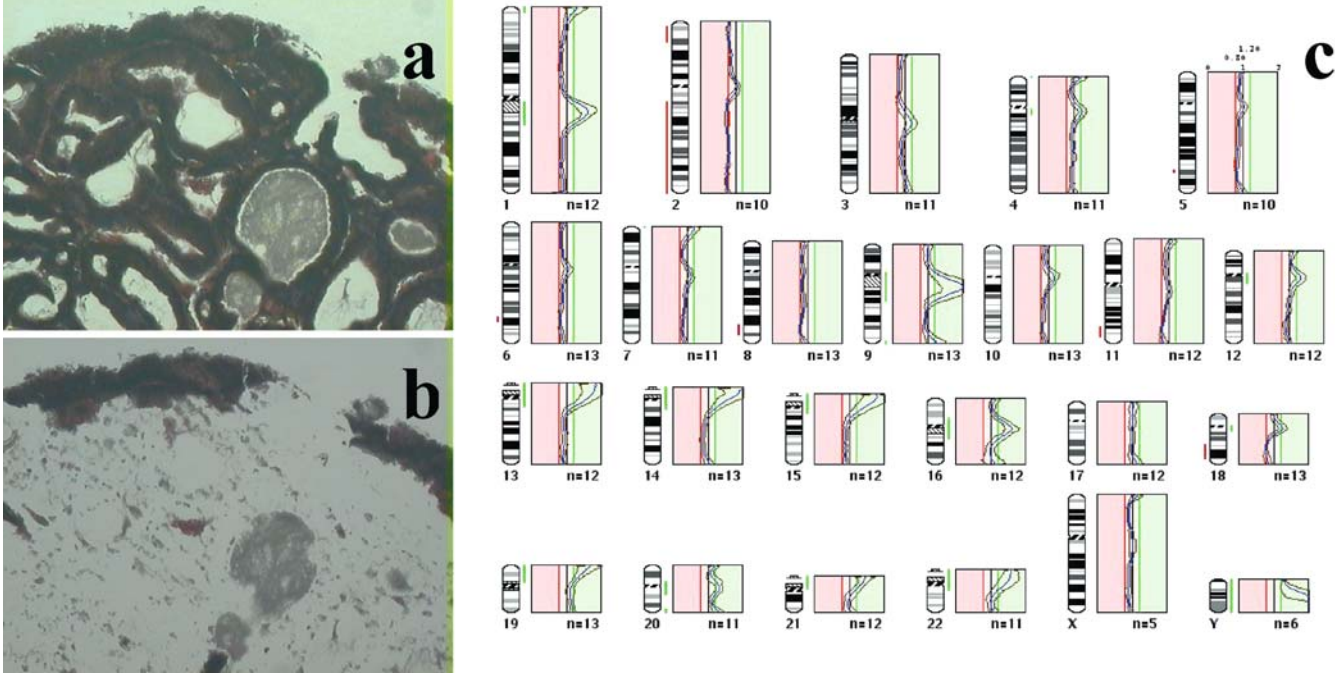

Fig. 4 Microdissection and comparative genomic hybridization (CGH) analyses of the tumor. **a** Pre-microdissection. **b** Post-microdissection. **c** CGH analysis shows losses on 2p24–25.2, 2q14.1-ter, 5q31.3–32, 6q23–24, 8q23–24.2, 11q22.3–24 and 18q21.1–22

USA), respectively, using random priming reaction. Gains and losses in DNA copy number were defined by green to red ratios (G/R) greater than 1.2 and less than 0.8, respectively. High-level gains (amplifications) were defined by a G/R greater than 1.5. Chromosomes 1p32-pter, 16p, 19, 22 and Y were excluded in the analyses [8].

Results

Pathological findings

A polypoid tumor, 2 mm in the longest diameter, was found to be surrounded by so-called specialized columnar epithelium (SCE), and two-layered muscularis mucosae was observed (Fig. 1). The tumor consisted of narrow or cystically dilated tubules. They were lined by cuboidal to prismatic columnar epithelial cells that contained clear cytoplasm or pale eosinophilic cytoplasm. Superficial tumor cells were slightly higher in size than deeper ones (Fig. 2a). The tumor cell nuclei were small and oval or round in shape and contained small but conspicuous nucleoli (Fig. 2b). These findings were identical to those described for pyloric gland adenoma of the stomach [24, 26] and some other digestive organs [10, 11, 14, 17].

Most tumor glands were strongly positive for MUC6, except in the superficial layer (Fig. 3a). MUC5AC was positive in almost all tumor cells (Fig. 3b), but MUC2 and CD10 were negative in the tumor. MIB-1-positive proliferating cells were distributed throughout the tumor (Fig. 3c). In the area of SCE surrounding the tumor, MUC5AC- and MUC6-positive cells tended to be located in the upper and lower portions of the glands, respectively. In the upper glands of SCE, there were scattered MUC2-positve goblet cells, most of which also expressed MUC5AC. CD10 positivity was focally found in the SCE area. Microdissection and CGH analyses revealed losses on 2p24–25.2, 2q14.1-ter, 5q31.3–32, 6q23–24, 8q23–24.2, 11q22.3–24 and 18q21.1–22 (Fig. 4).

Discussion

In our most recent clinico-pathological analysis of 90 cases of the pyloric gland adenoma, 15 cases (13%) were extragastric, but no esophageal case was encountered. Immediately after our publication, Michal et al. [15] described a case of pyloric gland adenoma underneath the normal squamous epithelium of the esophagus without BE. SCE of BE is identical to subtype of incomplete-type intestinal metaplasia of the stomach. Both metaplastic changes have widely been believed to be precancerous conditions, from which neoplasia of the gastric, gastrointestinal or intestinal phenotype may occur. Mucin histochemical analysis using mAbs against MUC2, MUC5AC and MUC6 recently revealed the mixed differentiation to intestinal and gastric phenotypes in SCE of BE and incomplete-type intestinal metaplasia of the stomach. Glickman demonstrated a higher frequency of MUC6 expression in SCE than in intestinal metaplasia of the stomach [4]. Therefore, it is very likely that the present tumor may have developed under induction of differentiation to pyloric gland-type cells in SCE of BE.

In the normal gastric mucosa or gastric metaplasia of extragastric mucosa, bi-directional differentiation of MUC5AC (apical part of the mucosa) and MUC6 (lower part of the mucosa) is displayed [12, 13]. However, the present tumor cells express both MUC6 and MUC5AC, except in the superficial layer, and MIB-1-positive cells were not localized as in intestinal-type adenomas [21]. Chromosomal aberrations found in the present tumor were partly common to those of Barrett's dysplasias, adenocarcinomas and gastric cardia adenocarcinomas [22, 23, 25]. These immunohistochemical and cytogenetic findings demonstrate that the tumor is immature, unstable and precancerous in nature. We recently demonstrated relatively high incidence of malignant transformation in pyloric gland adenomas [24]. Takei et al. [17] also showed a close association between pyloric gland adenomas and adenocarcinomas in the gallbladder. Pyloric gland adenoma of the gastric mucosa was first described by Elster [3] in 1976 but misinterpreted as adenoma-like hyperplasia of mucoid glands. In our experience, pyloric gland adenomas have been regarded as hyperplasia or hamartoma, and this entity has not been accepted, particularly in the Anglo-American literature, in the past. Endoscopists and pathologists must avoid misinterpretation and should be on the lookout for this type of tumor during routine examination.

In summary, we have presented the first case of pyloric gland adenoma arising in BE, showing its unstable and precancerous nature by mucin histochemical and cytogenetic methods.

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