

Single Case

Next-Generation Sequencing for Non-Ampullary Duodenal Carcinoma Suggesting the Existence of an Adenoma-Carcinoma Sequence

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Keywords

Familial adenomatous polyposis · Duodenal cancer · Adenoma-carcinoma sequence · Next-generation sequencing

Abstract

Duodenal tumors with a sporadic adenoma-carcinoma sequence are extremely rare. For such clinically suspected cases without a specific family history, performing a comprehensive gene search is important to understand the germline mutation background. We present a 68-year-old woman without a genetic or familial history of familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome, or Lynch syndrome who presented to Kosei Hospital, Japan, with exertional dyspnea induced by abdominal pain lasting 3 weeks. A duodenal tumor was suspected by contrast-enhanced computed tomography. Esophagogastroduodenoscopy showed a lesion accompanied by a white microprotuberance on the descending part of the duodenum

opposite the papilla, with a giant ulcerative lesion at the center of the white lesion. Biopsy revealed a low-grade adenoma, high-grade adenoma, and adenocarcinoma. Immunohistochemical analysis of the adenoma and adenocarcinoma showed Ki-67, p53, cytokeratin 20, caudal-type homeobox 2, and carcinoembryonic antigen positivity and cytokeratin 7 negativity. The findings suggested the presence of an adenoma-adenocarcinoma sequence in duodenal carcinoma. However, in the mutational analysis using next-generation sequencing, c.4348C>T (p.Arg1450Ter) mutation in APC was detected in all normal mucosal, adenoma, and carcinoma tissues. This mutation is common in FAP patients. Even if the presence of an adenoma-adenocarcinoma sequence in duodenal carcinoma is suggested in cases without a familial FAP history, as in this case, genetic analysis may reveal FAP. Thus, performing a comprehensive genetic analysis of duodenal carcinoma patients with a possible adenoma-carcinoma sequence is necessary to explore their genetic background.

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Introduction

The multistep theory of genetic mutation associated with the colorectal adenoma-carcinoma sequence is well documented [1, 2]. The adenoma-carcinoma sequence has been proposed to be established in ampullary duodenal carcinomas [3]. However, it is unclear whether the adenoma-carcinoma sequence also occurs in non-ampullary duodenal carcinomas. Relatively few studies have investigated morphological changes associated with the adenoma-carcinoma sequence in gastrointestinal carcinomas [4, 5].

Duodenal carcinoma in familial adenomatous polyposis (FAP) arises from adenomas. Differentially expressed genes in the duodenal adenoma-carcinoma pathway have been identified in murine FAP models; however, similar data in patients with FAP are limited [6]. Here, we report about a patient without a familial history of FAP in whom a histopathological finding suggestive of the adenoma-carcinoma sequence was obtained from the biopsy results of a bulging lesion in the duodenum, and the lesion was identified as an APC germline mutation by a genetic analysis.

Case Report

A 68-year-old woman without a genetic or familial history of FAP, Peutz-Jeghers syndrome, or Lynch syndrome presented to Kosei Hospital, Japan (Nakano, Japan) with exertional dyspnea induced by abdominal pain lasting 3 weeks. Contrast-enhanced computed tomography showed irregular wall thickening in the descending part of the duodenum (Fig. 1), and a heterogeneous contrast effect was observed at the same site. Multiple low-density areas were observed in the liver, suggesting a neoplastic lesion. Blood test results indicated that her carcinoembryonic antigen level was 73.5 ng/mL and her carbohydrate antigen 19-9 level was 112.2 U/mL, both of which exceeded the normal limits.

Esophagogastroduodenoscopy showed a lesion accompanied by a white microprotuberance on the descending portion of the duodenum opposite the papilla and a giant ulcerative lesion at the center of the white lesion. The range of the tumor is shown in Fig. 2 (dotted red line). The lesion site was different from that of the papilla, and it was judged to not be a papillary-derived lesion.

Given that ulcerative lesions exhibiting white elevated microprotrusions were observed, they were considered to be consecutive lesions. Biopsies of three consecutive lesions were performed (Fig. 2; red dotted lines). Biopsy No. 1 sample was taken from the white microprotruberance around the ulcer. Biopsy No. 2 sample was taken from the surrounding ridges accompanied by redness of the ulcer. Biopsy No. 3 sample was taken from the top of the ulcerative lesion to the contralateral side of the papilla.

Immunohistochemical Analysis

Biopsy samples were immunohistologically analyzed for the expression of p53 and Ki-67 (Fig. 3a). In lesion No. 1, p53- and Ki-67-expressing epithelial cells primarily formed a regular pattern at the base of the crypts. A higher proportion of Ki-67-expressing epithelial cells was found in lesion No. 2, and the cells were distributed in an irregular pattern. p53 was strongly expressed in epithelial cells, and the cells were regularly arranged. The proportion of Ki-67-expressing epithelial cells was the highest in lesion No. 3, and lesion No. 3 strongly expressed p53.

Based on pathology and immunohistochemistry, lesion No. 1 was considered to be a mild-to-moderate tubulovillous adenoma, whereas lesion No. 2 appeared to be a moderate-to-severe tubulovillous adenoma. Lesion No. 3 had both severe tubulovillous adenoma and well-to-poorly differentiated adenocarcinoma components. The tubular adenocarcinoma tested negative for cytokeratin (CK) 7 and positive for CK20 and caudal-type homeobox 2 (Fig. 3b). Based on the histopathological results, the patient was diagnosed as having a duodenal carcinoma and multiple liver metastases. Fluid replacement was performed for anorexia, which was considered to be because of the duodenal carcinoma. She was transferred to another hospital for chemotherapy after the diagnosis.

Immunohistochemistry was performed for human mutL homolog (MLH) 1, MSH2, MSH6, and postmeiotic segregation increased (PMS) 2 in all biopsy samples (Fig. 3c). The expression of MLH1, MSH2, MSH6, and PMS2 was observed in all tissues. Therefore, it was concluded that the patient did not have Lynch syndrome.

Based on the endoscopic and histopathological findings, the present case was considered to be a duodenal adenocarcinoma resulting in a duodenal carcinoma. We considered this to be a rare case that suggested an adenoma-carcinoma sequence in the duodenum. Therefore, we performed additional genetic tests to confirm the presence of an adenoma-carcinoma sequence in the duodenum.

Mutational Analysis by Next-Generation Sequencing

Genes were extracted from the cells of the normal mucosa, adenomas, and adenocarcinomas, and next-generation sequencing was performed for 50 genes. Mutational hotspots of 50 oncogenes and tumor-suppressor genes were analyzed using Ion AmpliSeq Carcinoma Hotspot Panel v2 (Life Technologies, Carlsbad, CA, USA) on an Ion Torrent Personal Genome machine (Ion PGM, Life Technologies). The following genes were analyzed: *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *BRAF*, *CDH1*, *CDKN2A*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB2*, *ERBB4*, *EZH2*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *GNA11*, *GNAS*, *GNAQ*, *HNF1A*, *HRAS*, *IDH1*, *JAK2*, *JAK3*, *IDH2*, *KDR*, *KIT*, *KRAS*, *MET*, *MLH1*, *MPL*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *PTPN11*, *RB1*, *RET*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *STK11*, *TP53*, and *VHL*.

The c.4348C>T (p.Arg1450Ter) mutation in *APC* was detected in all normal mucosal, adenoma, and carcinoma tissues. This mutation is common in FAP patients, and it is a pathogenic mutation [7, 8].

The c.659A>G (p.Tyr220Cys) mutation in TP53 was detected in patients with carcinomas only as somatic mutation. No *KRAS* mutations were found in any samples. Based on this, this case was confirmed to be a duodenal carcinoma occurring in an FAP patient with a germline APC mutation.

Outcome and Follow-Up

Distant metastases including liver metastases were recognized as unresectable factors. The patient was treated with chemotherapy at the hospital to which she was transferred. As first-line therapy, she was treated with 5-FU/l-LV/oxaliplatin (FOLFOX). The anti-tumor effect of FOLFOX was limited, and thus, she was treated with low-dose FP (cisplatin/5-fluorouracil) as the second-line therapy. Four months after initiating treatment, her general condition deteriorated, and the treatment was switched to palliative care. She died 5 months after initiating treatment.

Discussion

Our case highlights the occurrence of the adenoma-carcinoma sequence in a duodenal carcinoma based on histopathological evidence. In general, duodenal carcinomas account for 1% of all gastrointestinal carcinomas [9, 10]. Limited studies have described the adenoma-carcinoma sequence in the duodenum, which is a carcinogenic variant. The present case had no characteristics, such as a family history, suggesting an inherited disease; however, in such cases, it is necessary to confirm the presence of an inherited disease, such as FAP and Lynch syndrome. Therefore, in addition to histopathological evaluations, we performed a comprehensive gene analysis using next-generation sequencing. A subsequent genetic analysis revealed that the patient harbored a germline APC mutation [11]. Although the existence of the adenoma-carcinoma sequence in colon carcinomas of FAP patients is well-documented, the existence of the adenoma-carcinoma sequence in duodenal carcinomas of FAP patients remains controversial [12, 13].

There are multiple possibilities for the primary origin of carcinomas of the duodenum, such as duodenal, pancreatic, bile duct, and papillary carcinomas. Additionally, primary carcinoma of the duodenum can have various causes. In particular, in duodenal adenocarcinomas with duodenal adenomas, it is extremely difficult to diagnose duodenal carcinomas owing to FAP in patients without a family history of FAP. Previous reports have suggested the presence of the adenoma-carcinoma sequence in small intestine carcinomas [14]. However, there are also reports that the adenoma-carcinoma sequence in small intestine carcinomas is extremely rare [15].

This case of an advanced duodenal adenocarcinoma in which an APC germline mutation was detected using next-generation sequencing, which has the characteristic of the adenoma-carcinoma sequence, is important. Thus, a comprehensive gene analysis is important for rare cases having a possibility of the presence of the adenoma-carcinoma sequence in duodenal carcinomas.

Statement of Ethics

This study was approved by the Research Ethics Committee of Kosei Hospital, Japan. Informed consent for publication including images was obtained from the patient's family. Research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yohei Kojima cooperated as an instructor. Takashi Nikaido and Kouki Ohtsuka provided technical support and advice. Ryusuke Shimada, Hidesato Tanabe, Koichi Tabei, Osamu Yanagida, Hiroaki Ohnishi, Nobutsugu Abe, Tadakazu Hisamatsu, and Shinichi Takahashi provided advice.

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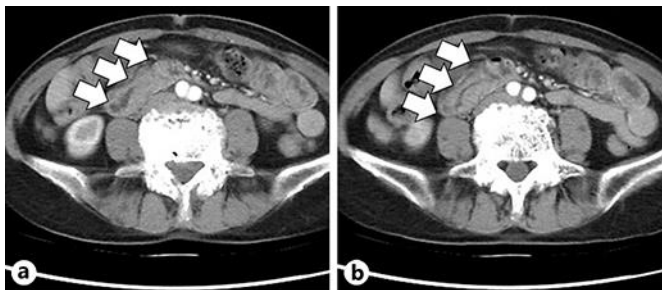


Fig. 1. Contrast-enhanced computed tomography. The two images show the height of the descending portion of the duodenum. The image on the right (**b**) is located 5 mm closer to the head than the image on the left (**a**). The descending portion of the duodenum showed circumferential wall thickening (arrows), with the head on the opposite side of the papilla of the neck, suggesting a neoplastic lesion.

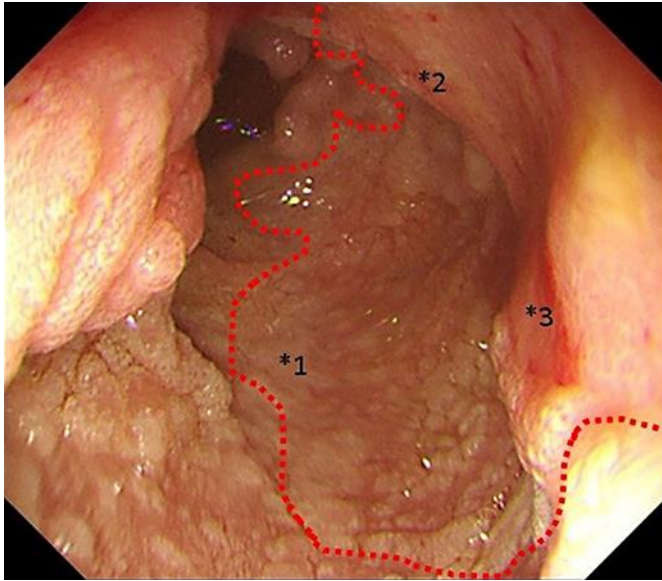


Fig. 2. Upper gastrointestinal endoscopy. Biopsies were performed at site No. 1–3. All biopsies were performed using samples from a series of lesions surrounded by the dotted red line. Biopsy No. 1 sample was taken from the white microprojections around the ulcer. Biopsy No. 2 sample was taken from the surrounding ridge with ulcerative redness. Biopsy No. 3 sample was taken from the top of the ulcerative lesion on the other side of the nipple.

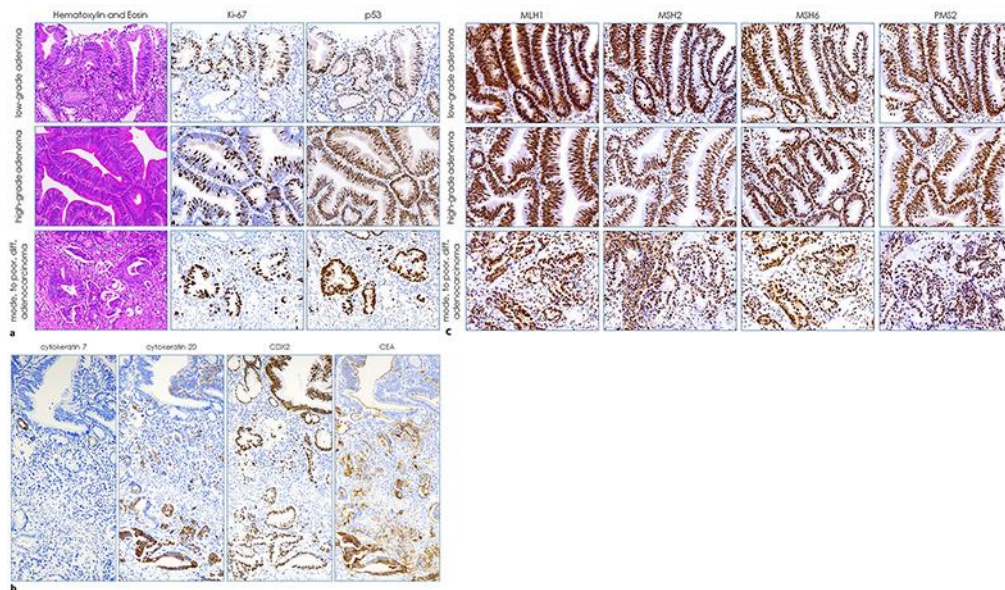


Fig. 3. Pathology and immunostaining results. **a** Microscopic analysis of the tumor. The upper row shows the results of biopsy No. 1, the middle row shows biopsy No. 2, and the lower row shows biopsy No. 3. The left column displays hematoxylin and eosin (H&E) staining, the middle column shows expression of Ki-67, and the right column shows expression of p53. Biopsy No. 1 was diagnosed as a low-grade adenoma, biopsy No. 2 as a high-grade adenoma, and biopsy No. 3 as a moderate-to-poorly differentiated adenocarcinoma (200×). **b** To rule out the possibility of metastasis or invasion from other primary sites, immunohistochemistry was performed for cytokeratin 7, cytokeratin 20, caudal-type homeobox (CDX) 2, and carcinoembryonic antigen (CEA). The tissue tested negative for cytokeratin 7 and positive for cytokeratin 20, CDX2, and CEA (100×). **c** Immunohistochemistry for human mutL homolog (MLH) 1, MSH2, MSH6, and postmeiotic segregation increased (PMS) 2 was performed in all three biopsy samples. All of these proteins were expressed, indicating that the patient did not have Lynch syndrome (200×).