Identification of \textit{APC} Gene Mutations in Jejunal Carcinomas from a Patient with Familial Adenomatous Polyposis

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Jejunal carcinoma in patients with familial adenomatous polyposis has been rarely reported, and little is known about its association with genetic alterations of the \textit{APC} gene. A 52-year-old woman with familial adenomatous polyposis underwent palliative resection of the proximal jejunum because of two circumferential tumors associated with peritoneal carcinomatosis. A histological examination revealed that one tumor was a poorly differentiated adenocarcinoma, and that the other was a moderately differentiated adenocarcinoma with adenomatous components. The patient did not respond to standard chemotherapy and died of disseminated disease 8 months after surgery. A genetic analysis of the \textit{APC} gene identified somatic mutations in each tumor (c.4450delAG and p.R1450X) in addition to the germline mutation (c.3984del5), all of which form stop codons, resulting in truncated \textit{APC} products. This report is the first description of how a second hit to the \textit{APC} gene can be involved in carcinogenesis of the jejunum in familial adenomatous polyposis.

Key words: familial adenomatous polyposis – jejunal carcinoma – \textit{APC} gene – mutation

INTRODUCTION

Classical familial adenomatous polyposis (FAP) is an autosomal-dominant inherited disease caused by a germline mutation of the \textit{APC} gene. The disease is characterized by the formation of more than 100 adenomatous polyps in the colon and rectum. The adenoma–cancer sequence in FAP has been well established, and nearly 100% of patients with FAP will develop colorectal cancers by their fourth decade if untreated. With the widespread use of a prophylactic colectomy, the number of deaths caused by colorectal cancer has decreased (1), and one of the current leading causes of death for reasons other than colorectal cancer is duodenal carcinoma (1,2) originating from duodenal adenomas. With the use of various endoscopic techniques, polyps in the small intestine which are located distal to the duodenum can be identified in up to 75% of patients (3–5). These polyps are mostly small (<5 mm), low-grade dysplastic adenomas in the proximal jejunum (3–5). Although the clinical significance of such small lesions is unclear, high-grade dysplastic adenomas and carcinomas in the jejunum can occur. We report an extremely rare case of double carcinomas of the proximal jejunum occurring in an FAP patient and present a review of the literature. We also identified the germline and somatic alterations of the \textit{APC} gene in these rare tumors.

CASE REPORT AND GENETIC ANALYSIS

CASE REPORT

A 52-year-old woman was admitted to our hospital complaining of upper abdominal pain. She had undergone a total proctocolectomy with an ileal-pouch anal anastomosis for diffuse colonic polyposis at the age of 20 years at another hospital. She had also undergone 10 additional laparotomies for adhesiolysis, the resection of intra-abdominal desmoid tumors or...
the resection of duodenal polyps, although sufficient information on each treatment could not be obtained. Her mother had died of colon cancer associated with FAP at the age of 36 years. Her son had also been diagnosed as having FAP at the age of 19 years. Her aunt, uncle and grandmother also had died of colon cancer at their early decade, even though we could not obtain sufficient data on the presence of colonic polyposis (Fig. 1). Laboratory data upon admission revealed almost normal findings. Her serum carcinoembryonic antigen level was <0.5 ng/ml. An abdominal computed tomography examination demonstrated a mass lesion in the proximal jejunum. A small-bowel X-ray examination showed severe stenosis distal to the Treitz ligament. An upper intestinal endoscopy revealed multiple duodenal polyposis, but the scope could not reach the distal third of the duodenum. A small-intestinal endoscopy was scheduled, but upper gastrointestinal decompression was necessary 7 days after admission. Therefore, she underwent a laparotomy 18 days after admission without a definitive diagnosis but with a strong suspicion of a malignant tumor. Upon laparotomy, marked adhesions were observed between the bowel tracts. Numerous peritoneal carcinomatosis and two circumferential tumors were also observed in the upper jejunum. One tumor was located 5 cm (Tumor 1) and the other tumor was located 20 cm (Tumor 2) distally from the Treitz ligament. A segmental resection of the proximal jejunum was performed (Fig. 2A and B). She underwent a gastrojejunostomy for a stricture of the duodenojejunostomy 21 days after the resection of the proximal jejunum, but an anastomotic leak subsequently occurred. Oxaliplatin-based chemotherapy (modified FOLFOX6) was started 2 months later once the anastomotic leak had almost resolved with conservative treatment. After six cycles of mFOLFOX6, multiple pulmonary metastases developed. Irinotecan-based chemotherapy (FOLFIRI) was started as a second-line treatment, but the patient died of disseminated disease 9 months after the jejunal resection. An autopsy was not performed.

A histological examination revealed double carcinomas: Tumor 1 was a poorly differentiated adenocarcinoma (pT3) (Fig. 3A), with a diameter of 80 mm, while Tumor 2 was a moderately differentiated adenocarcinoma (pT3) with adenomatous components (Fig. 3B) and a diameter of 50 mm. Multiple tubular adenomas were also found. Mesenteric lymph node metastases near Tumor 1 were also identified.

**GENETIC ANALYSIS**

A genetic analysis of the *APC* gene was approved by the local ethics committee of Saitama Medical University. After genetic counseling, the patient provided a written informed consent for a genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes using a standard procedure, and was also extracted from the frozen tissue samples of the tumors. A germline mutation in the *APC* gene was identified from the blood samples by direct sequencing of the PCR-amplified segments using standard methods. For the tumor tissue samples, direct sequencing of PCR-amplified genomic fragments of exons 1–15 was performed. All the nucleotide numbers refer to the wild-type complementary DNA sequence of *APC*, as reported in GenBank (accession no. NM_000038). The types of mutations were described according to the nomenclature for the description of sequence variants (http://www.hgvs.org/mutnomen/).

Two *APC* mutations were detected in Tumor 1 (c.3927del5 and c.4393delAG) (Fig. 4A) and in Tumor 2 (c.3927del5 and c.4393delAG) (Fig. 4B).

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**Figure 1.** Pedigree of the patient. The arrow (P) indicates the proband. Filled symbols indicate affected individuals. d = died; op = operation.

**Figure 2.** (A) Macroscopic appearance of Tumor 1, which was located 5 cm from the Treitz ligament. The tumor has a locally ulcerating shape. (B) Macroscopic appearance of Tumor 2, which was located 30 cm from the Treitz ligament. The tumor has a protruding shape.
p.R1450X (Fig. 4B). The APC mutation was also detected in the blood sample (c.3927del5). Thus, we identified the germline mutation as c.3927del5 and the somatic mutations as c.4393delAG and p.R1450X. This type of germline mutation has been already identified (http://www.insight-group.org/mutations/). All the APC mutations that were detected were predicted to form stop codons, resulting in truncated APC products. In c.3927del15, a frameshift mutation consisting of a 5 bp deletion from nucleotide 3927 (codon 1309) in exon 15 resulted in a stop codon at codon 1312. c.4393delAG consists of a deletion of nucleotides 4393–4394 at codon 1465, leading to a stop codon at codon 1467 in exon 15. p.R1450X indicates the replacement of C with T at the first nucleotide in codon 1450, changing arginine to a stop codon.

DISCUSSION

The incidence of jejunal/ileal carcinoma among FAP patients has not yet been fully investigated. According to the data collected from 10 registries, Jagelman et al. (6) reported that the incidence of carcinoma of the jejunum and ileum were 0.4% (5 of 1255) and 0.1% (1 of 1255), respectively. Based on the data from the Japanese polyposis registry, Iwama et al. (2) reported that jejunal/ileal carcinoma accounted for 1.0% of all causes of death among FAP patients. To our knowledge, seven case reports of eight jejunal carcinomas associated with FAP, including our case, have been published in English journals between 1980 and 2012 (7–10) (Table 1). These cases consisted of 5 men and 2 women, with a median age of 52 years (range 34–71 years) at the time of diagnosis of the jejunal carcinoma(s). The interval between (procto)colectomy and the diagnosis of jejunal carcinoma ranged from 7 to 42 years (median, 20 years). The type of colorectal polyposis was the classical form in six cases (including diffuse polyposis) and the attenuated form (11) in one. All the five documented cases were associated with duodenal polyposis. Multiple jejunal polyps were identified in all the three documented cases. The histological diagnoses included mucinous carcinoma in one tumor and adenocarcinoma in the other seven tumors. Four of the seven patients had Stage IV disease, and their prognoses were poor.
Table I. Collected cases of jejunal carcinoma(s) associated with familial adenomatous polyposis reported in the English literature between 1980 and 2012

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Age (years)</th>
<th>sex</th>
<th>Form of colorectal polyposis</th>
<th>Interval since (procto) colectomy (years)</th>
<th>Duodenal polyps</th>
<th>Jejunal polyps</th>
<th>Histology</th>
<th>pTNM stage</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillips (1981)</td>
<td>34</td>
<td>Male</td>
<td>Classical (typical)</td>
<td>12</td>
<td>Not documented</td>
<td>(+)</td>
<td>Adenocarcinoma</td>
<td>stage IV</td>
<td>Died of disease</td>
</tr>
<tr>
<td>Zuidema and Dekkar (1989)</td>
<td>39</td>
<td>Male</td>
<td>Classical (typical)</td>
<td>13 (++)</td>
<td>Not documented</td>
<td>(+)</td>
<td>Mucinous carcinoma</td>
<td>stage I</td>
<td>Died of disseminated disease (11 months)</td>
</tr>
<tr>
<td>Eigenbrod et al. (9)</td>
<td>51</td>
<td>Female</td>
<td>Attenuated</td>
<td>7</td>
<td>Not documented</td>
<td>Not documented</td>
<td>Moderately diff. adenocarcinoma</td>
<td>stage II</td>
<td>Survived metastatectomy and chemotherapy (&gt; 36 months)</td>
</tr>
<tr>
<td>Ruys et al. (2010) (10)</td>
<td>71</td>
<td>Male</td>
<td>Classical (typical)</td>
<td>23</td>
<td>(+)</td>
<td>Not documented</td>
<td>Moderately diff. adenocarcinoma with tubulovillous adenoma</td>
<td>stage IV?</td>
<td>Alive and disease-free (4 months)</td>
</tr>
<tr>
<td>Ruys et al. (2010) (10)</td>
<td>57</td>
<td>Male</td>
<td>Classical (typical)</td>
<td>20</td>
<td>(+)</td>
<td>Not documented</td>
<td>Adenocarcinoma</td>
<td>stage IV</td>
<td>Died of disseminated disease (within 12 years)</td>
</tr>
<tr>
<td>Ruys et al. (2010) (10)</td>
<td>59</td>
<td>Male</td>
<td>Classical (typical)</td>
<td>7</td>
<td>(+)</td>
<td>Not documented</td>
<td>Adenocarcinoma</td>
<td>stage IV</td>
<td>Died of disseminated disease</td>
</tr>
<tr>
<td>Present case</td>
<td>52</td>
<td>Female</td>
<td>Classical (typical)</td>
<td>42</td>
<td>(+)</td>
<td></td>
<td>(1) Poorly diff. adenocarcinoma (2) Moderately diff. adenocarcinoma with tubulovillous adenoma</td>
<td>stage IV</td>
<td>Died of disseminated disease (8 months)</td>
</tr>
</tbody>
</table>

FAP, familial adenomatous polyposis.
Because of the widespread use of a prophylactic (procto) colectomy, the incidence of death caused by colorectal cancer in FAP patients has been decreasing (1). Patient management should now focus on surveillance for duodenal lesions in patients who have undergone a prophylactic (procto) colectomy, since duodenal or periampullary carcinoma, together with desmoid tumors, is a leading cause of death among FAP patients (1, 2). Duodenal polyposis has been reported in 30–90% (12–14) of FAP patients, and a surveillance protocol according to the Spiegelman classification (15) has been established. In contrast, the need for surveillance in the jejunum and ileum has been questioned since the development of carcinomas in the jejunum/ileum is extremely rare and evidence supporting the removal of jejunal/ileal polyps, usually <5 mm (3–5), is lacking. Polyps (adenomas) are reportedly detected more frequently in the jejunum than in the ileum (3–5). The carcinoma–adenoma sequence can be extrapolated for jejunal tumorigenesis. In addition, patients with duodenal polyposis tend to have more jejunal/ileal polyps, compared with those without duodenal lesions (4, 16). Based on these characteristics together with the reportedly poor prognosis of FAP patients who develop jejunal carcinoma, efforts to detect jejunal carcinoma at an early stage using a small-bowel endoscopy in selected patients, such as those with severe duodenal polyposis and a relatively advanced age as shown in our literature review (Table 1), may deserve further investigations.

With respect to the site of the germline mutation and the number of colorectal adenomas in FAP patients, genotype–phenotype correlations have been well described. Profuse polyposis is known to be associated with germline mutations located between codons 1250 and 1464 (17). Some germline mutations are related to the presence of extracolonic manifestations: for example, desmoid tumors are associated with germline mutations between codons 1445 and 1560 (18). Our case had profuse polyposis and intra-abdominal desmoid tumors. Regarding profuse colorectal polyposis, the germline mutation in our case (c.3927del5) concurs with the previous findings. To the best of our knowledge, Ruys et al. (10) first identified the germline mutation (W157X) in exon 4 in a 59-year-old man with Stage IV jejunal carcinoma. Whether specific mutation sites in patients with FAP might be associated with jejunal carcinoma remains unclear, and further data collection is needed.

In general, a tumor suppressor gene is inactivated through a germline mutation and a somatic mutation or loss of separate alleles, resulting in tumor formation in accordance with Knudson’s ‘two-hit’ model. However, the APC gene in tumors of FAP patients does not entirely follow this model. The locus of the second mutation is influenced by the status of first germ-line mutation with a residual number of 20-amino acid (20-AA) repeats that are thought to be critical for β-catenin binding and degradation, affecting intracellular β-catenin levels. This mechanism has been called ‘the just-right signaling model’ (19) and is considered to explain tumorigenesis in FAP, at least in part. Miyaki et al. (20) reported that as a result of the germline mutation and the loss of the wild-type allele, 96% of the colorectal tumors associated with profuse-type FAP retained only the first 20-AA repeat located at codons 1265–1284 in a unilateral allele of APC, and 69% of the colorectal tumors associated with sparse-type FAP retained the first two 20-AA repeats at codons 1265–1284 and 1379–1398. These findings suggest that one or two residual 20-AA repeats, which regulate the β-catenin level in a sensitive manner, influence the phenotype of FAP. The number of β-catenin downregulating 20-AA repeats associated with tumorigenesis also differs between colorectal and extracolonic tumors, such as gastric, duodenal and desmoid tumors.

To the best of our knowledge, the number of retained 20-AA repeats has not been investigated in jejunal/ileal tumors associated with FAP. The authors previously identified a somatic and a germline mutation in a jejunal adenoma that caused intussusception in a male FAP patient (21). Based on the sites of mutations (germline mutation: 4 bp deletion at codons 181 and 182, somatic mutation: T insertion in codon 1557), the total number of 20-AA repeats retained was three. In the present case, the germline mutation retained one 20-AA repeat. In addition, the somatic mutations in Tumors 1 and 2 each retained two 20-AA repeats. Therefore, the total number of retained 20-AA repeats was three in each tumor. These findings seem to represent the first documentation of both germ-line and somatic mutations in the APC gene, with special attention given to the number of retained 20-AA repeats, in jejunal carcinomas associated with FAP. The collection of further data is needed to investigate jejunal tumorigenesis based on ‘the just-right signaling model’ (19).

Authors’ contribution
All authors contributed to this work. Hideyuki Ishida designed the study and wrote the manuscript; Kensuke Kumamoto performed genetic analysis and genetic counseling; Kensuke Kumamoto and Keiichi Ishibashi prepared the description of the clinical course; Morihiro Higashi and Junichi Tamura made a histological diagnosis.

Conflict of interest statement
None declared.

References