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Single Case

STK11 p.F354L Germline Mutation in a Case of Multiple Gastrointestinal Tumors

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Keywords

STK11 · p.F354L · Duodenal adenoma · Gastric adenoma · Colorectal adenocarcinoma · Liver metastasis

Abstract

Serine/threonine kinase 11 (*STK11*) is known as a critical tumor-suppressor gene that is frequently mutated in a broad spectrum of human cancers. Among these, the p.F354L mutation of *STK11* has been identified in sporadic colon or lung cancer cases. Here, we report the case of a 75-year-old male patient who underwent surgical treatment for multiple tumors of the gastrointestinal system. Genetic mutations were screened in all resected samples, including duodenal high-grade adenoma, gastric high-grade adenoma, rectal adenocarcinoma, and liver metastasis of rectal adenocarcinoma, by next-generation sequencing for mutational hotspots involving 50 oncogenes and tumor suppressor genes. The characteristic hamartomatous polyp of Peutz-Jeghers syndrome was not detected in any tumor specimen. However, all samples as well as the normal rectal mucosa harbored the genetic mutation p.F354L in *STK11*. In addition, somatic mutations coexisted in the tumor samples, including *KRAS* p.A146T, *TP53* p.G238X, and *APC* p.T1556fs in the duodenal adenoma; *TP53* p.G238Y and *APC* p.T1556fs in the gastric adenoma; and *TP53* p.R282W in the rectal adenocarcinoma and metastatic liver cancer. No somatic mutation was detected in the normal rectal mucosa as a control sample. To our

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knowledge, this is the first report of an *STK11* germline mutation in a patient with multiple tumors of the gastrointestinal tract.

Introduction

Mutation in serine/threonine kinase 11 (*STK11*), also known as liver kinase B1 (*LKB1*), was first identified as the causal mutation in Peutz-Jeghers syndrome (PJS), a rare inherited autosomal dominant disorder characterized by the development of benign gastrointestinal hamartomas and the early onset of cancer [1]. Since then, *STK11* has become recognized as a critical tumor-suppressor gene that is frequently mutated in a broad spectrum of human cancers.

STK11 directly phosphorylates and regulates adenosine monophosphate-activated protein kinase [2, 3]. In particular, the *STK11* p.F354L mutation has been reported in cases of sporadic colon cancer [4]. We also reported that the *STK11* p.F354L mutation may play a key role in the development of duodenal adenoma and adenocarcinoma [5].

Here, we report the first case of a patient with multiple tumors (stomach, duodenum, and rectum) of the gastrointestinal tract harboring an *STK11* germline mutation without specific PJS findings.

Case Report

A 75-year-old man presented to another hospital with anemia. Gastrointestinal endoscopies revealed gastric, duodenal, and rectal tumors, and the patient was referred to our hospital for treatment. The patient had not been diagnosed with PJS previously. His family members did not have any signs or symptoms related to PJS. Esophagogastroduodenoscopy demonstrated a protruding tumor (12 mm in diameter) on the lesser curvature side of the gastric body (Fig. 1b) and another protruding tumor (20 mm in diameter) in the duodenal bulb (Fig. 1a). Endoscopic punch biopsies of the tumors revealed a gastric high-grade adenoma and a duodenal high-grade adenoma, respectively. Furthermore, colorectal endoscopy revealed an ulcerated tumor in the rectum (Fig. 1c), and endoscopic punch biopsy revealed an adenocarcinoma. An abdominal CT (computed tomography) scan revealed neither regional lymph node swelling nor lung/liver metastases.

The gastric and duodenal adenomas were resected by endoscopic submucosal resection. The rectal cancer was resected by laparoscopic high-anterior resection. Histopathologically, the duodenal (Fig. 3a) and gastric (Fig. 3b) tumors were both graded as high-grade dysplasia adenoma based on the World Health Organization tumor classification system according to differences in the atypical ductal structure, nuclear atypia, and cytoplasm/nucleus ratio. The rectal tumor was histologically diagnosed as a well-differentiated adenocarcinoma (Fig. 3c). This tumor invaded the rectal muscularis propria with fibrosis. Moderate lymphatic and venous invasion were observed. One lymph node metastasis was diagnosed. According to the TNM staging system, the rectal cancer stage was IIIA. Histologically, these tumors (stomach, duodenum, and rectum) exhibited no characteristic feature of the hamartomatous polyp of PJS.

A hepatic tumor was detected at the 1-year follow-up by enhanced CT scanning (Fig. 2). The lesion was found in segment 8 of the hepatic area with a size of 3 cm, and was surgically resected. Histologically, the hepatic tumor was diagnosed as a metastatic adenocarcinoma

from rectal cancer (Fig. 3d). This patient has had no recurrence for more than 5 years after the hepatic surgery without any chemotherapy.

Subsequently, we carried out molecular analyses by next-generation sequencing [5] to screen for genetic mutations in the tumor samples and a normal rectal mucosa sample as the control (Fig. 3e). Genomic DNA was extracted from formalin-fixed paraffin-embedded sections (10 μm) of all samples using the ZR FFPE DNA Miniprep kit (Zymo Research, Irvine, CA, USA) in accordance with the manufacturer's protocol. DNA integrity was examined by amplification of control genes using real-time PCR (polymerase chain reaction) with the QuantStudio 6 Flex Real-Time PCR system (Thermo Fisher Scientific), producing fragments of approximately 200 bp [6, 7]. Samples with DNA integrity equal to or higher than 150 pmol were selected for analysis of mutational hotspots by next-generation sequencing.

To analyze the mutational hotspots of 50 oncogenes and tumor suppressor genes, we used the Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies, Carlsbad, CA, USA) on an Ion Torrent Personal Genome machine (Ion PGM; Life Technologies); the genes analyzed were *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*. We performed sequencing on a 318 chip using a Sequencing kit 200 v2 (Thermo Fisher Scientific) according to the manufacturer's protocols. Signal processing, mapping, and quality control were performed with Torrent Suite v4.2 (Thermo Fisher Scientific). Sequence variants were called using Ion Reporter v4.2 (Thermo Fisher Scientific) and the AmpliSeq CHPv2 single-sample workflow with default settings. Variants were subsequently filtered to include only exonic, nonsynonymous variants with allelic frequencies higher than 10%. A sequence variation was considered to be a potential mutation when the coverage was above 100× and the allelic frequency was above 10%. The samples were examined using SIFT and Polymorphism Phenotyping v2 (PolyPhen-2); a SIFT score of <0.05 indicates that the substitution is carcinogenic, and PolyPhen-2 scores in the range of 0.15–0.85 suggest that the substitution is carcinogenic, while scores of 0.85–1.0 indicate that the substitution is likely carcinogenic [8, 9].

All samples harbored the same mutation: *STK11* p.F354L. Somatic mutations coexisted in the tumor samples, including *KRAS* p.A146T, *TP53* p.G238X, and *APC* p.T1556fs in the duodenal adenoma, and *TP53* p.G238Y and *APC* p.T1556fs in the gastric adenoma. Therefore, the same *APC* and *TP53* mutations were detected in the duodenal adenoma and gastric adenoma. The rectal adenocarcinoma and liver metastasis had the common somatic mutation *TP53* p.R282W. The rectal mucosa harbored only the mutation *STK11* p.F354L. No somatic mutation was detected in the normal rectal mucosa as the control sample.

Moreover, microsatellite instability (MSI) was only tested in the duodenal high-grade adenoma sample. The MSI status was assessed within common microsatellite locations (BAT25, BAT26, NR21, NR24, and NR27), which revealed no deficient mismatch repair phenotype.

Discussion

The germline mutation *STK11* p.F354L was detected in a case of multiple tumors (gastric high-grade dysplasia adenoma, duodenal high-grade dysplasia adenoma, and rectal adenocarcinoma) of the gastrointestinal tract by next-generation sequencing-based screening of more than 2,800 mutational hotspots among 50 oncogenes and tumor suppressor genes [5]. Next-generation sequencing revealed the *STK11* p.F354L mutation in all samples (including

metastatic adenocarcinoma of the rectal cancer) coexisting with somatic mutations, including the control sample.

The point mutation *STK11* p.F354L has previously been reported in cases of lung cancer or PJS [10–13]. Our patient had no familial history or typical characteristics and symptoms of PJS. Therefore, this is the first report of a patient with multiple primary tumors, including adenoma, associated with this *STK11* mutation. Moreover, this is the first report of a comprehensive screening effort with next-generation sequencing in a case of multiple tumors, including high-grade dysplasia adenoma, revealing an *STK11* germline mutation.

In vitro analyses showed that *STK11* with the p.F354L mutation activates AMPK-like kinases, thereby enhancing the proliferation of cells transfected with these mutations [10–12]. Notably, p.F354L was recently reported as a single nucleotide polymorphism prevalent in the Japanese population at frequencies of 1–3% (UCSC Genome Bioinformatics). Moreover, the ClinVar database from the National Library of Medicine classifies this point of mutation as benign/likely benign. However, we previously reported that *STK11* mutation was confirmed in 25% of duodenal adenocarcinomas and 9% of duodenal adenomas, which are higher rates than the reported prevalence in the Japanese population [5]. Therefore, our study suggests that the *STK11* p.F354L mutation should be further validated considering its potential key role in the development of duodenal adenoma and adenocarcinoma.

Schabath et al. [14] reported the presence of commonly co-occurring mutations in the *STK11* or *TP53* tumor suppressor genes in lung cancer, which were suggested to confer tumorigenic ability in *KRAS*-mutant tumors. Mutation in *TP53* was strongly associated with enhanced proliferation, and *STK11* mutation was associated with the suppression of immune surveillance [14]. The same mechanisms of tumorigenicity may also be involved in gastrointestinal tumors, although this hypothesis requires further direct testing [5]. We also detected somatic mutations coexisting in the same tumor. *KRAS*, *TP53*, and *APC* mutations were detected in the duodenal adenoma, and the *TP53* p.R282W mutation was detected in both the rectal adenocarcinoma and metastatic liver cancer. Therefore, these combinations of genetic mutations may affect tumorigenesis not only in the lung but also in the gastrointestinal tract.

One of the key findings of our case was that there was the same *STK11* mutation in multiple gastrointestinal tract tumors. Hemminki et al. [1] suggested the possibility that *STK11* may not only be a tumor suppressor gene that regulates hamartoma formation but might also play an important role as an early gatekeeper in the hamartoma-adenoma-carcinoma sequence. However, we did not detect a hamartoma in the gastrointestinal tract in the present case; therefore, validating this hypothesis requires further accumulation of cases associated with *STK11* mutation.

MSI may also be present in these tumors and could play important roles in their development [6]. However, MSI was only tested in the duodenal high-grade adenoma sample and was negative. Therefore, MSI is considered to be very unlikely to have played a role in the tumorigenesis of this case.

In summary, we reported an unusual case of multiple tumors of the gastrointestinal tract associated with an *STK11* germline mutation. Based on comprehensive molecular analysis, we suggest that the germline mutation of *STK11* p.F354L may cause gastrointestinal tumors, including adenoma, and we also found coexisting somatic mutations of *TP53*, *APC*, and *KRAS* in the tumors of this patient. This case suggests that in a patient with *STK11* mutations without symptoms of PJS, the carcinogenesis pathway may involve development of adenocarcinomas due to malignant transformation from adenoma. Further functional studies are needed to establish the precise role of *STK11* in cancer development of the gastrointestinal tract.

Statement of Ethics

This study was approved by the Research Ethics Committee of Kyorin University Faculty of Medicine. Informed consent was obtained from the patient.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Y. Kojima proposed the concept and wrote the manuscript. K. Ohtsuka provided technical support and advice. H. Ohnishi supervised the laboratory work. S. Ishii, N. Aso, A. Ohki, Y. Hashimoto, and H. Takeuchi provided advice. N. Abe led the surgery and study design and drafted the manuscript.

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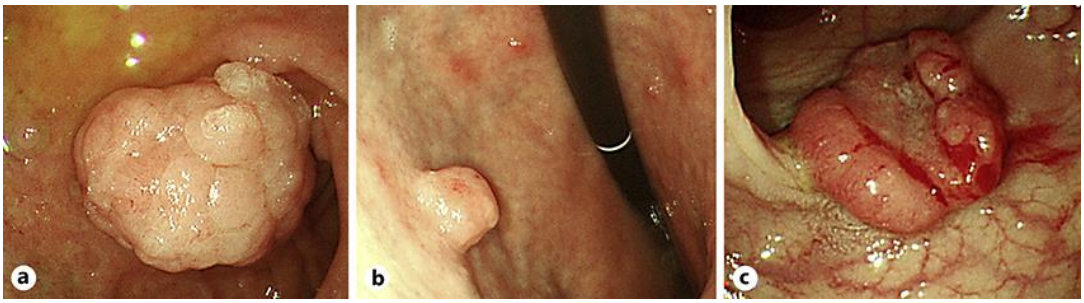

Fig. 1. Endoscopic findings. **a** Duodenal polyp (2 cm) on the bulb portion with a protruded shape. **b** Gastric polyp (1.2 cm) with a protruded shape on the anterior wall of the gastric body. **c** Rectal adenocarcinoma (5 cm) with an ulcerative region.

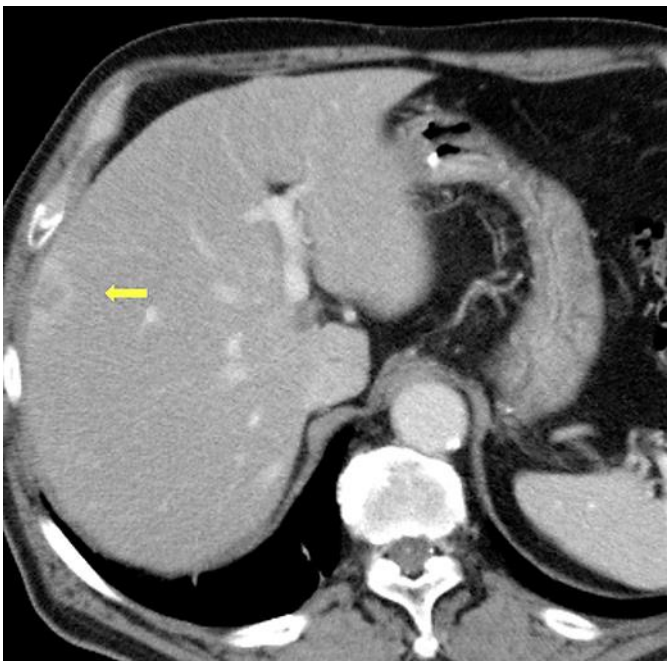

Fig. 2. Radiographic imaging. Computed tomography scan of the liver tumor of 3 cm. The bulls eye symbol (arrow) in this lesion indicates a low-density area surrounded by a high-density capsule.

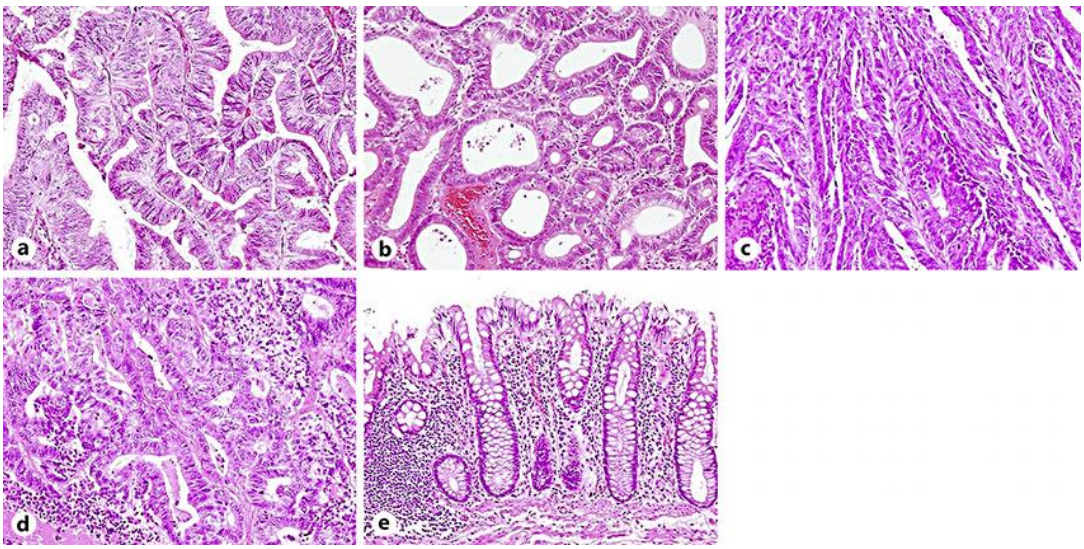

Fig. 3. Representative histopathological images of each lesion with hematoxylin-eosin staining. **a** Duodenal tumor with high-grade dysplasia glands (×200). **b** Gastric tumor with high-grade dysplasia glands (×200). **c** Rectal tumor with glands of adenocarcinoma (×200). **d** Liver tumor with glands of adenocarcinoma similar to rectal adenocarcinoma (×200). **e** Rectal normal mucosa analyzed as a control sample (×100).