Human Pathology (2014) 45, 884–888

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Human PATHOLOGY

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Case study

Multiple gastrointestinal stromal tumors with novel germline c-*kit* gene mutation, K642T, at exon 13☆

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Received 12 July 2013; revised 8 November 2013; accepted 8 November 2013

Keywords:
GIST;
Familial;
Germline mutation;
Exon 13;
Heterotopic ossification

Summary Multiple gastrointestinal stromal tumors (GISTs) caused by germline c-*kit* gene mutations are an extremely rare autosomal dominant disorder. A 57-year-old Japanese woman was referred to a hospital for appetite loss and severe weight loss. She had 2 large abdominal masses around the stomach, which were surgically resected. Histological examination revealed that these tumors were GISTs. Multiple microscopic GISTs and diffuse hyperplasia of the interstitial cells of Cajal were also seen in the background gastric and small intestinal walls. Characteristically, the GISTs showed severe hyalinization with calcification and partial heterotopic ossification, which may have caused the patient's severe dysphagia. Mutational analysis of the c-*kit* gene revealed a substitution at codon 642 in exon 13 (K642T) in the tumor, normal ileal mucosa and peripheral blood leukocytes, indicating that the mutation is in the germline. This is the first case of multiple GISTs with novel germline c-*kit* gene mutation at exon 13.

1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the human digestive tract [1]. Most sporadic GISTs are caused by the constitutive activation of KIT, a type III receptor tyrosine kinase, which is encoded by the *KIT* (c-*kit*) gene located on chromosome 4q12 and composed of 21 coding regions [2]. The mechanism of activation in most sporadic GISTs is an alteration of the structure of the receptor's extracellular or cytoplasmic domains caused by somatic mutations of the c-*kit* gene, and this change leads to dimerization and autophosphorylation of KIT with subsequent activation of signal transduction cascades in the absence of ligand binding [2]. Inhibition of KIT activity by a specific tyrosine kinase inhibitor, imatinib, often results in dramatic clinical responses [1].

In contrast to GISTs associated with somatic mutations, GISTs caused by germline mutations are extremely rare. To our knowledge, there have been 26 reports of familial GISTs associated with germline mutations of the c-*kit* gene [3-9]. In addition to multiple GISTs, affected families might exhibit a variety of clinical symptoms including hyperpigmentation,

☆ Conflicts of interest and source of funding: the authors have disclosed that they have neither conflicts of interest nor sources of funding related to this article.

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0046-8177/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.11.009

urticaria pigmentosa and dysphagia [5]. Because of the limited number of families with proven germline c-*kit* gene mutations, it has still not been determined whether different locations or types of germline c-*kit* gene mutation are associated with specific phenotypes.

We here report one additional case with a germline c-*kit* gene mutation at exon 13, K642T, which has not yet been described as either a somatic or a germline mutation. In addition, the patient had severe dysphagia, and the GISTs in the digestive tract showed characteristically prominent hyalinization and calcification with heterotopic ossification. We also discuss these symptoms and phenotypes associated with the novel germline mutation found in this patient.

2. Case report

A 57-year-old woman was admitted to a local hospital complaining of difficulty eating and weight loss of 20 kg during the past year. Gastric endoscopic examination showed extrinsic compression in the upper body of the stomach. The patient was then followed as an outpatient. One and a half years later, she was taken to Aizawa Hospital, Matsumoto, Japan, via ambulance because of loss of consciousness. Blood sugar was as low as 9 mg/dL and severe weight loss (−10 kg) had occurred in the past month. Computed tomography scan disclosed two large tumors around the gastric upper body with severe calcification around the gastric cardia (Fig. 1). She underwent total gastrectomy. During the operation, multiple small nodules were observed on the subserosa of the small intestine. Therefore, partial resection of the small bowel including the nodules was added. After the pathological diagnosis, imatinib administration was initiated (200 mg/d) at 49 days after the operation, but unfortunately this had to stop after 28 days of administration because of liver dysfunction. Computed tomography scan disclosed slight progression of the residual small tumors 226 days after the operation. Nine months after the operation, the patient is alive with an outpatient status. She has difficulty eating, probably because of the thickened wall of the lower esophagus at the proximal side of the esophago jejunum anastomosis.

3. Materials and methods

3.1. Immunohistochemical analysis

Surgically resected specimens were fixed in 10% formalin for one day and embedded in paraffin. Three-micrometer-thick sections were stained with hematoxylin-eosin. Immunohistochemical staining was performed using an automated immunostainer (Ventana Benchmark XT; Ventana Medical Systems, Inc, Tucson, AZ). Antibodies against CD117 (c-kit, polyclonal; 1:200; DAKO, Glostrup, Denmark), CD34 (QBEND10; 1:1; Beckman Coulter, Marseille, FR), S-100 protein (polyclonal; 1:2000; DAKO), alpha-smooth muscle actin (1A4; 1:400; DAKO) and Ki-67 (MIB-1; 1:200; DAKO) were used in the present study.

3.2. Sequence analysis of the c-*kit* gene

For mutational analysis, genomic DNA was extracted from paraffin-embedded sections of the tumor tissue and normal ileal mucosa tissue using QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Genomic DNA was also extracted from peripheral blood leukocytes of the patient using the same kit. Exons 9, 11, 13 and 17 of the c-*kit* gene were amplified by polymerase chain reaction (PCR). The primers used for the PCR were as described previously [3]. Direct sequencing of the PCR products was carried out with ABI BigDye Terminator ver. 3.1 (Applied Biosystems, Foster City, CA) and ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). Informed consent was obtained, and the mutational analyses were approved by institutional review boards.

4. Results

4.1. Histopathology and immunohistochemistry

Macroscopically, cut surfaces of the two gastric tumors were white to grayish and partly yellowish. They had maximum diameters of 8.0 and 5.5 cm. Yellow spotted pigments were observed on the serosal side of the gastric upper body to the fornix. Around the upper body and the cardia, the gastric wall was thickened and hard. Microscopically, the two large tumors were composed of spindle-shaped cells, which were strongly positive for both CD117 and CD34 and negative for both S-100 protein and alpha-smooth muscle actin by immunohistochemistry (Fig. 1A and B). These tumors showed ~1 mitosis/50 high-power fields and ~1% MIB-1 labeling index. Prominent hyalinization and calcification were found in parts of the tumors. In the upper body of the stomach, hyalinization and calcification were more prominent, and partial heterotopic ossifications with bone marrow formation were observed (Fig. 1D and E). Many small nodules were also seen in the background gastric wall, suggesting that the lesions involved hyperplasia of interstitial cells of Cajal (ICCs) in the stomach (Fig. 1C).

Multiple small nodules in the subserosa of the resected small bowel were also composed of spindle cells positive for both CD117 and CD34, indicating that the lesions were multiple GISTs. Moreover, CD117-positive diffuse ICC hyperplasia was observed at the myenteric plexus layer within the muscularis propria (Fig. 2).

4.2. Mutational status of the patient

Gastric GISTs of the patient had a K642T mutation at exon 13 of the c-*kit* gene. Normal ileal mucosa without GIST and