

# Clinicopathologic study of succinate-dehydrogenase-deficient gastrointestinal stromal tumors

## A single-institutional experience in China

Weizhen Liu, PhD<sup>a</sup>, Xiangyu Zeng, MM<sup>a</sup>, Xiuli Wu, MM<sup>b</sup>, Jun He, MM<sup>b</sup>, Jinbo Gao, MD<sup>a</sup>, Xiaoming Shuai, MD<sup>a</sup>, Guobin Wang, MD, PhD<sup>a</sup>, Peng Zhang, MD<sup>a,\*</sup>, Kaixiong Tao, MD, PhD<sup>a,\*</sup>

### Abstract

Gastrointestinal stromal tumors (GISTs) that are not driven by kinase mutations, as are most GISTs, often show loss of function of the succinate dehydrogenase (SDH) complex and are considered SDH-deficient GISTs. SDH-deficient GISTs share many distinct characteristics compared with conventional GISTs. However, data regarding these characteristics, particularly among Asian people, are relatively limited. The objective of this study was to characterize the clinicopathologic characteristics, treatment, and prognosis of these uncommon GISTs.

This retrospective observational study enrolled 12 patients with SDH-deficient GISTs, who were selected from 335 patients with GIST diagnosed at our institution between October 31, 2013 and October 31, 2016 by succinate dehydrogenase subunit B staining.

There were 8 male and 4 female patients, with a median age of 57 years (range, 21–73 years). Ten patients (83.3%) were diagnosed at or after the age of 40 years and represented 7.2% (10/138) of the entire population of elderly patients with gastric GISTs. The tumor size ranged from 3 to 19 cm (median, 7 cm); the primary tumor was multifocal in 6 cases (50%), and tumors had a multinodular or plexiform architecture in 10 cases (83.3%). Ten cases (83.3%) showed pure epithelioid morphology, with the remaining 2 cases (16.7%) showing mixed histologic subtype. Lymph node metastasis was found at the time of primary resection in 50% (3/6) of patients. Four cases (33.3%) had distant metastasis at presentation. Four patients (33.3%) developed disease progression during imatinib treatment after initial resection, but all of these patients regained disease control when the treatment was altered to sunitinib targeted therapy.

SDH-deficient GISTs arise exclusively in the stomach and account for approximately 7.4% (12/162) of gastric GISTs. Moreover, those affecting people older than 40 years are not uncommon and sunitinib may work well for cases showing treatment failure with imatinib.

**Abbreviations:**  $\alpha$ KG =  $\alpha$ -ketoglutarate, BVI = blood vessel invasion, CSS = Carney–Stratakis syndrome, CTr = Carney triad, GI = gastrointestinal, GISTs = gastrointestinal stromal tumors, HIF-1 $\alpha$  = hypoxia-inducible factor 1 alpha, HPFs = high-power fields, IGF = insulin-like growth factor, IHC = immunohistochemistry, NIH = National Institutes of Health, PAGFRA = platelet-derived growth factor alpha, SDH = succinate dehydrogenase, SDHB = succinate dehydrogenase subunit B, TET = ten-eleven translocation, VEGF = vascular endothelial growth factor, WT = wild type.

**Keywords:** gastrointestinal stromal tumors, prognosis, SDHB, succinate dehydrogenase, succinate dehydrogenase subunit B, targeted therapy

Editor: Laszlo Geza Boros.

WL and XZ contributed equally to this work.

**Funding:** This study was supported by National Key Basic Research Program of China (2015CB5540007), National Natural Science Foundation of China (81572413), the Fundamental Research Funds for the Central Universities (2017KFYXJJ256), Scientific and Technological Application Foundation Project of Wuhan (2015060101010044), and the Scientific Research Training Program for Young Talents of Wuhan Union Hospital.

The authors have no conflicts of interest to disclose.

<sup>a</sup>Department of Gastrointestinal Surgery, <sup>b</sup>Department of Pathology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

\*Correspondence: Peng Zhang, Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan, Hubei Province 430022, China (e-mail: zhangpengwh@hust.edu.cn); Kaixiong Tao, Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan, Hubei Province 430022, China (e-mail: kaixiongtao@hust.edu.cn).

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

Medicine (2017) 96:32(e7668)

Received: 19 April 2017 / Received in final form: 5 July 2017 / Accepted: 7 July 2017

<http://dx.doi.org/10.1097/MD.0000000000007668>

## 1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract, with an estimated annual incidence of 14 to 20 per million people.<sup>[1,2]</sup> The great majority of GISTs harbor activating mutations of *KIT* (75–80%) or platelet-derived growth factor receptor alpha (*PDGFRA*) (5–8%).<sup>[2]</sup> However, approximately 15% of GISTs in adults and more than 90% of pediatric GISTs lack these tyrosine kinase mutations and are generally classified as “wild-type” (WT) GISTs.<sup>[3]</sup> Recent studies have shown that WT GISTs are quite heterogeneous in terms of clinical phenotype, genetic etiology, and molecular pathways.<sup>[4,5]</sup> Among them, succinate dehydrogenase (SDH)-deficient GISTs, which are associated with SDH deficiency by immunohistochemistry (IHC), are the largest group.

SDH, which is located in the inner mitochondrial membrane, is involved in the fundamental processes of energy production by participating in the electron transport chain (complex II) and catalyzing the oxidative dehydrogenation of succinate to fumarate in the Krebs cycle.<sup>[6]</sup> This complex consists of 4 subunit proteins (SDHA, succinate dehydrogenase subunit B [SDHB], SDHC, and SDHD), all of which are entirely encoded by chromosomal DNA. Mutational inactivation or loss of any SDH component (A, B, C, or D) leads to the loss of SDHB expression due to a destabilization of and subsequent loss of function of the SDH complex, and therefore, SDHB IHC can be used as a surrogate to identify these GISTs.<sup>[7]</sup> SDH-deficiency causes the truncation of the Krebs cycle, which leads to metabolic reprogramming of mitochondrial respiration, and sustained malignant proliferation of glucose and fatty acids.<sup>[8]</sup> In addition, SDH-deficiency contributes to succinate accumulation, and its pathologic elevation creates a “pseudohypoxic” state, which then triggers the hypoxia-inducible factor 1 alpha (HIF-1 $\alpha$ )-mediated hypoxia response that supports tumor formation by activating angiogenesis.<sup>[9]</sup> Furthermore, due to the structural similarities between succinate and  $\alpha$ -ketoglutarate ( $\alpha$ KG), succinate accumulation is thought to inhibit  $\alpha$ KG-dependent dioxygenase enzymes, such as the ten-eleven translocation (TET) family of DNA hydroxylases.<sup>[10]</sup> TET proteins convert 5-methylcytosine to 5-hydroxymethylcytosine, which is essential for subsequent DNA demethylation. Therefore, succinate accumulation due to SDH deficiency could potentially drive tumorigenesis via the inhibition of TET family proteins and subsequently alter global DNA methylation; this might thereby influence gene expression.

*KIT/PDGFRA*-mutated GISTs can occur anywhere in the GI tract, have an equal sex distribution, usually show a spindle morphology, rarely metastasize to lymph nodes or distant organs and frequently respond to imatinib. By contrast, a small number of reports have determined that SDH-deficient GISTs are located exclusively in the stomach, show a predilection for children and young adults, have a female preponderance, and are characterized by a distinctive multinodular/plexiform architecture and an epithelioid or mixed histologic subtype.<sup>[11]</sup> Occasional cases of SDH-deficient GISTs show symptoms related to those of metastatic tumors in the liver or abdomen. Furthermore, SDH-deficient GISTs run a relatively indolent course despite their frequent lymph node or distant metastasis and exhibit consistent primary resistance to imatinib therapy.

Due to the rarity of SDH-deficient GISTs and the mostly recent interest in these tumors, data regarding SDH-deficient GISTs, particularly within Asian populations, are relatively limited. In this study, we performed SDHB IHC on 335 GISTs diagnosed at

our institution from October 31, 2013 to October 31, 2016, ultimately selecting 12 cases of SDH-deficient GISTs, aiming to characterize the clinicopathologic characteristics, treatment, and prognosis of these uncommon GISTs.

## 2. Materials and methods

### 2.1. Study design, patients, and setting

Of 842 patients with GISTs who were evaluated since 2005 at our clinic, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology in China, the data of 335 patients who were diagnosed between October 31, 2013 and October 31, 2016 were retrospectively evaluated and included in the study. This retrospective study was approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology according to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, and written informed consent was obtained from all 335 study participants.

### 2.2. Tumor samples and clinicopathologic data

For each case, hematoxylin and eosin and IHC slides and pathology reports were reviewed by 2 experienced GI pathologists. Data concerning tumor location, maximal tumor diameter, multifocality, multinodular (plexiform) architecture, morphologic subtype, necrosis, lymphovascular invasion, lymph node metastasis, and mitotic figures per 50 high-power fields (HPFs) were recorded. Detailed clinical history, including history of genetic testing and clinical follow-up, were collected from medical records, outpatient recheck, and telephone interviews with physicians. The tumors were classified into prognostic risk groups according to the modified NIH stratification criteria.<sup>[12]</sup>

### 2.3. Succinate dehydrogenase-B immunohistochemistry

IHC for SDHB was performed on 4-mm-thick formalin-fixed paraffin-embedded whole tissue sections using commercially available mouse monoclonal antibodies directed against SDHB (1:100 dilution; 40 minutes incubation; clone 21A11AE7; Abcam, Cambridge, MA) following pressure cooker antigen retrieval (Target Retrieval Solution, pH 6.1; Dako, Carpinteria, CA), as previously described.<sup>[2,3]</sup> Cases showing definite granular cytoplasmic staining were classified as positive. Cases not showing cytoplasmic staining in the presence of an internal positive control of nonneoplastic cells (e.g., vascular, smooth muscle, and epithelial elements) were classified as negative. If the findings of 2 pathologists were different or uncertain (e.g., due to weak or absent internal positive controls), IHC was repeated on a whole section and a third pathologist was consulted.

## 3. Results

### 3.1. Baseline features

Of the 335 total GISTs cases, 162 arose primarily from the stomach, among which 12 cases (7.4%) of SDH-deficient GISTs were identified. The clinical and pathologic characteristics of the patients are summarized in Table 1. There were 8 male and 4 female patients in our study (male/female ratio of 2:1), with a median age of 57 years (range, 21–73 years). Of note, 10 patients (83.3%) were diagnosed at or after the age of 40 years, and the remaining 2 patients were diagnosed at 21 and 24 years,

**Table 1**  
**Clinicopathological characteristics of 12 patients with SDH-deficient GISTs.**

| Characteristic                             | N (%)     |
|--|-----------|
| Age, y                                     |           |
| <40  | 2 (16.7)  |
| ≥40  | 10 (83.3) |
| Sex  |           |
| Male                                       | 8 (66.7)  |
| Female                                     | 4 (33.3)  |
| Location (gastric)                         |           |
| Corpus                                     | 5 (41.7)  |
| Antrum                                     | 5 (41.7)  |
| Fundus                                     | 2 (16.6)  |
| Tumor size (mean; range), cm               | 7 (3–19)  |
| Distant metastasis at presentation         | 4 (33.3)  |
| Liver                                      | 3 (75)    |
| Peritonea                                  | 1 (25)    |
| Association (Carney triad)                 | 1 (8.3)   |
| Mitotic rate per 5mm <sup>2</sup> (median) | 2–18 (6)  |
| Cytology                                   |           |
| Epithelioid                                | 10 (83.3) |
| Mixed                                      | 2 (16.7)  |
| Multifocal                                 | 6 (50)    |
| Multinodular                               | 10 (83.3) |
| Tumor necrosis                             | 9 (75)    |
| Blood vessel invasion                      | 3 (25)    |
| Lymph node metastasis (n=6)                | 3 (50)    |
| IHC  |           |
| CD117 positive                             | 12 (100)  |
| CD34 positive                              | 11 (91.7) |
| DOG-1 positive                             | 11 (91.7) |
| SMA positive                               | 4 (33.3)  |
| S-100 positive                             | 2 (16.7)  |
| Ki-67 (mean, range)(%)                     | 3 (1–30)  |
| SDHB deficient                             | 12 (100)  |
| Genetic analysis (WT)(n=7)                 | 7 (100)   |

GISTs=gastrointestinal stromal tumors, IHC=immunohistochemistry, SDHB=succinate dehydrogenase subunit B, WT=wild type.

respectively; the 10 patients represented 7.2% (10/138) of the entire population of elderly patients with gastric GISTs. All 12 cases of SDH-deficient GISTs were located in the stomach, including 5 (41.7%) in the corpus, 5 (41.7%) in the antrum, and 2 (16.6%) in the fundus. One patient (8.3%) was presumed to have a Carney triad (CTr) due to the asynchronous occurrence of a pulmonary chondroma. Of the 12 SDH-deficient patients, 4 (33.3%) had metastasis at the time of the initial diagnosis: 3 (75%) involved metastasis to the liver (Fig. 1A and B) and 1 (25%) to the peritoneum. Three of the 4 metastatic masses were removed along with the primary tumor, and the remaining case of metastasis was not removed because of its deep location in liver segment I.

### 3.2. Pathologic characteristics

The tumor size varied from 3 to 19 cm (median, 7 cm). The mitotic rate varied from 2 to 18 per 50 HPFs (median mitotic count, 6/50 HPFs). Ten cases (83.3%) showed pure epithelioid morphology (Fig. 2A), with the remaining 2 cases (16.7%) showing a mixed histologic subtype (Fig. 2B). Six cases of primary tumors (50%) were multifocal (Fig. 1C and D), with 10 cases (83.3%) showing a multinodular or plexiform architecture (Fig. 2C). Nine cases (75%) had tumor necrosis. Lymphovascular invasion was observed in 3 (25%) cases (Fig. 2D), and lymph

node metastasis were encountered in 3 of 6 cases (Fig. 2E). Of the total 162 cases of GIST located in the stomach, most (150, 92.6%) were immunohistochemically positive for SDHB (Fig. 2F), with granular cytoplasmic staining of various intensities. IHC for SDHB staining of the 12 SDH-deficient GISTs showed loss of expression (Fig. 2G and H). Furthermore, all cases had positive CD117, CD34, and DOG-1 expression, with negative SMA and S-100 expression. In addition, genetic analysis was performed in 7 patients and that all had WT GISTs.

### 3.3. Treatment and follow-up

All 12 cases underwent surgical resection, and the detailed interventions are shown in Table 2. Follow-up data for all patients were available, ranging from 9 to 34 months (mean 21 months), with no deaths. According to modified NIH stratification criteria, 9 patients were at high-risk, 1 at moderate-risk, and 2 at low-risk. Of the 10 patients at high- or moderate-risk, 9 were administered targeted therapy (imatinib or sunitinib). Four patients experienced disease progression during imatinib treatment after the initial resection, but all of them showed progression-free survival when the treatment was changed to sunitinib targeted therapy. The remaining patients who were administered targeted therapy had a favorable prognosis.

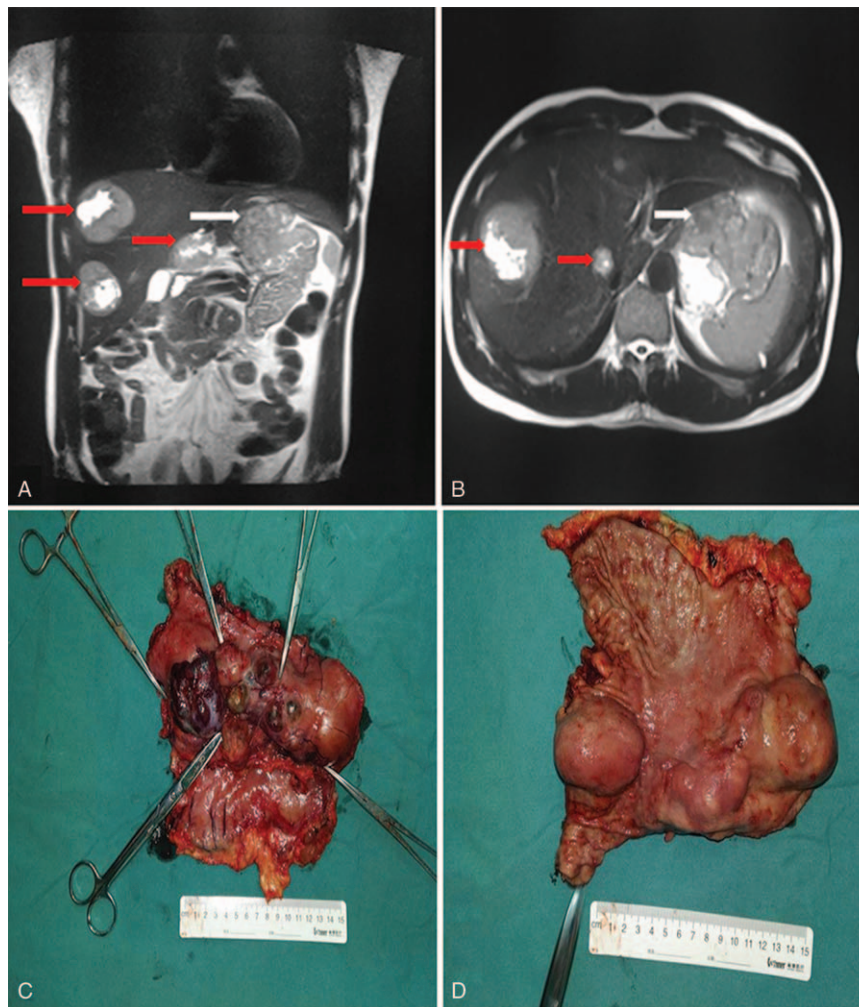
## 4. Discussion

SDH-deficient GISTs represent the largest proportion of the 10% to 15% of GISTs in adults and the 90% in children that lack *KIT* or *PDGFRA* mutations, and they are often grouped together and considered as WT GISTs.<sup>[13]</sup> Although several studies in America and Europe have revealed the clinicopathologic and IHC features and prognosis of SDH-deficient GISTs, the data in Asian populations remains relatively limited. Herein, we presented the first study of SDH-deficient GISTs described in China. In this study, we examined SDHB levels by IHC in 335 primary GISTs treated at our institution within the recent 3 years and demonstrated that SDHB staining was absent in 7.4% (12/162) of gastric GISTs. Similarly, the rate that Miettinen et al<sup>[14]</sup> predicted based on their cohort of 66 SDH-deficient GISTs was 7.5%. In addition, the largest study (76 cases) of SDH-deficient GISTs conducted by Mason and Hornick<sup>[15]</sup> showed a frequency of approximately 7.7%.

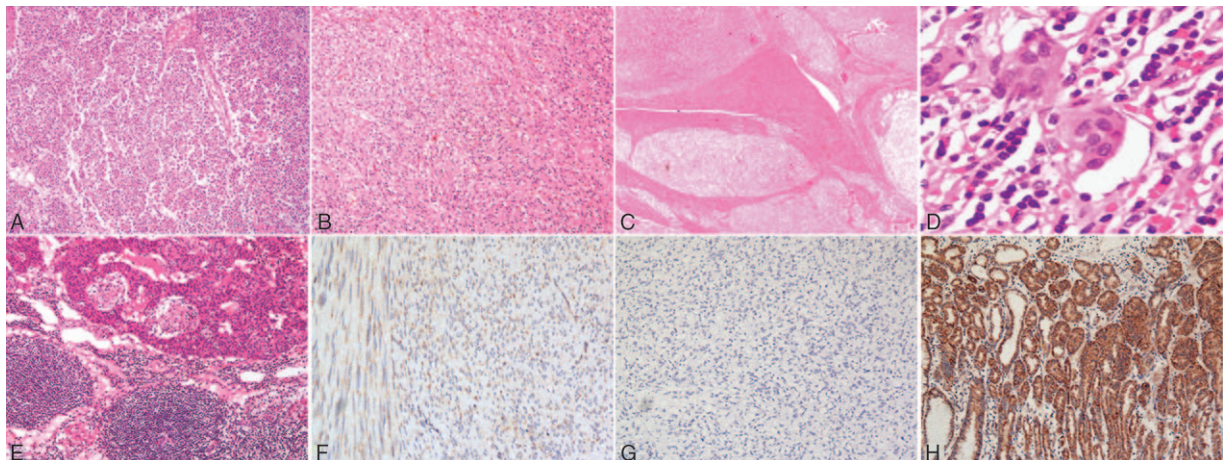
Previous studies have demonstrated that SDH-deficient GISTs were more likely to occur in younger, female patients. Mason and Hornick<sup>[15]</sup> reported a median age of 32 and a male/female ratio of 1:1.5. This finding conflicts with the present study, where an overwhelming majority of patients (10/12, 83.3%) were diagnosed at or after the age of 40 years, representing 7.2% (10/138) of the study population of elderly patients with gastric GISTs. Furthermore, there were 8 male and 4 female patients, resulting in a male/female ratio of 2:1. The apparent bias in this ratio might be in part due to the small number of cases. However, the large proportion of elderly patients may suggest that SDH-deficient GISTs affecting elderly people are not uncommon.

In addition to sporadic SDH-deficient GISTs, a small subset of patients with SDH-deficient GISTs fulfills criteria for 1 of 2 tumor syndromes, Carney–Stratakis syndrome (CSS) or CTr.<sup>[16]</sup> CTr is a nonhereditary syndrome involving gastric GISTs along with pulmonary chondroma and extraadrenal paraganglioma, with a predominance in young female.<sup>[17]</sup> CSS is a hereditary syndrome characterized by the occurrence of GISTs and paragangliomas. Unlike CTr, CSS has an equal sex distribution and more





**Figure 1.** SDH-deficient GISTs (A and B, white arrows) are common with live metastasis (A and B, red arrows) at the time of initial diagnosis and often show multifocal gastric masses (C and D).



**Figure 2.** Representative histological and IHC features of SDH-deficient GISTs. Tumors present epithelioid (A, H&E,  $\times 100$ ) or mixed epithelioid and spindle cell (B, H&E,  $\times 100$ ) morphology and characteristically show a multinodular or plexiform architecture (C, H&E,  $\times 20$ ). Lymphovascular invasion in the primary resection (D, H&E,  $\times 400$ ) and lymph node metastasis (E, H&E,  $\times 100$ ) are common findings. Traditional GISTs with SDHB expression (F, IHC staining,  $\times 100$ ). SDH-deficient GISTs present completely negative for SDHB immunostaining in tumor cells (G, IHC staining,  $\times 100$ ) but positive in mucosal cells (H, IHC staining,  $\times 100$ , positive internal control).

**Table 2****Manifestation, treatment, and follow-up of 12 patients with SDH-deficient GISTs.**

| Sex/age, y | Manifestation                         | Distant metastasis at presentation | Intervention  | Risk     | Targeted therapy                        | Follow-up   |
|------------|---------------------------------------|------------------------------------|---|----------|---|---|
| M/50       | Hematochezia                          | No                                 | Proximal gastrectomy  | High     | No                                      | RFS at 34 mo  |
| M/57       | Hematochezia                          | No                                 | Partial gastrectomy with cauda pancreatis, spleen, and liver mass | High     | Imatinib (400 mg/d) → sunitib (50 mg/d) | Liver metastasis at 8 mo from initial resection → sunitib (50 mg/d) → PFS at 26 mo  |
| F/21       | Hematochezia, dizziness, and fatigue  | Yes (peritonea)                    | Subtotal gastrectomy with metastasis mass                         | High     | Imatinib (400 mg/d)                     | RFS at 30 mo  |
| M/60       | Dysphagia                             | No                                 | Wedge gastrectomy   | High     | Imatinib (400 mg/d)                     | RFS at 29 mo  |
| M/73       | Abdominal distension and vomiting     | No                                 | Total gastrectomy   | High     | Imatinib (400 mg/d) → sunitib (50 mg/d) | Initial resection → imatinib (400 mg/d) → local recurrence at 10 mo → resection → sunitib (50 mg/d) → RFS at 24 mo            |
| M/67       | Upper abdominal pain and hematochezia | No                                 | Total gastrectomy with spleen                                     | High     | Imatinib (400 mg/d)                     | RFS at 29 mo  |
| M/69       | Upper abdominal pain                  | No                                 | Wedge gastrectomy   | Low      | No                                      | RFS at 21 mo  |
| F/60       | Upper abdominal pain                  | Yes (liver)                        | Wedge gastrectomy with liver mass                                 | Low      | No                                      | RFS at 18 mo  |
| M/52       | Upper abdominal pain                  | No                                 | Wedge gastrectomy   | High     | Imatinib (400 mg/d)                     | RFS at 19 mo  |
| F/67       | Abdominal distension and fatigue      | No                                 | Wedge gastrectomy   | Moderate | Imatinib (400 mg/d)                     | RFS at 15 mo  |
| M/40       | Upper abdominal pain                  | Yes (liver)                        | Proximal gastrectomy  | High     | Imatinib (400 mg/d) → sunitib (50 mg/d) | Initial diagnosis → resection → imatinib (400 mg/d) → disease progression in liver at 5 mo → sunitib (50 mg/d) → PFS at 11 mo |
| F/24       | Hematemesis                           | Yes (liver)                        | Distal gastrectomy with liver mass                                | High     | Imatinib (400 mg/d) → sunitib (50 mg/d) | Initial diagnosis → imatinib (400 mg/d) → disease progression at 6 mo → resection → sunitib (50 mg/d) → RFS at 9 mo           |

GISTs=gastrointestinal stromal tumors, mo=months, PFS=progression-free survival, RFS=recurrence-free survival, SDH=succinate dehydrogenase.

commonly occurs in elderly patients. Both of these syndromes are underrecognized because of the rare asynchronous occurrence of the different tumor components in the same individual. Particularly in CTr, paragangliomas often occur in a long span time apart from GISTs and only 25% of the patients have all 3 tumors; therefore, the presence of any 2 of the components is sufficient for the diagnosis.<sup>[18]</sup> In our review, 1 female patient had CTr with pulmonary chondroma diagnosed at 21 years, which was the youngest observed at our institution. Recently, Boikos et al<sup>[19]</sup> reported that of the 95 total patients in their study, 9 (9.5%) had incomplete CTr, 7 (77.8%) of whom were female, with a median age of 21 years (range, 13–37 years).

Although we were only able to analyze 12 cases, our findings suggested that SDH-deficient GISTs arose exclusively in the stomach, showing epithelioid (10/12) or mixed (2/12) morphology, multifocal disease (6/12), and common lymph node metastasis (3/6), whereas these features were extraordinarily rare in conventional *KIT/PAGFRA*-mutant GISTs. Similarly, the rate of lymph node metastasis reported by Miettinen et al<sup>[14]</sup> was 41.7% (5/12) and that reported by Boikos et al<sup>[19]</sup> was 51% (18/35). Moreover, blood vessel invasion (BVI) was detected in 3 cases (25%) in the present study. Yamamoto et al<sup>[20]</sup> found that BVI was a strong indicator of liver metastasis in GIST and they noted that when BVI was present in the primary localized GIST, approximately 80% of patients subsequently developed liver metastasis. Interestingly, the 1 patient who developed liver metastasis at 8 months after the initial resection in our observations was found to have BVI in the tumor sample. Not surprisingly, IHC examinations for all cases showed positivity for CD117, CD34, and DOG-1 and negativity for SMA and S-100. All cases with an available genetic analysis in our study (7/12) exhibited WT, which is consistent with the results of previous investigations.<sup>[16,17]</sup>

Loss of SDHB expression is a consistent feature of SDH-deficient GISTs, whereas SDHB expression is intact in conven-

tional GISTs.<sup>[21]</sup> Approximately half of SDH-deficient GISTs have *SDH* subunit gene mutations, often germline and most commonly A (30%), and B, C, or D (together 20%), with both alleles inactivated in the tumor cells according to the classic tumor suppressor gene model.<sup>[22]</sup> However, the remaining half of SDH-deficient GISTs lack identifiable *SDHx* mutations; therefore, the mechanism of inactivation of SDH in those cases remains unclear. In 2014, Killian et al<sup>[23]</sup> uncovered a recurrent gene silencing epimutation of *SDHC* highly specific to SDHx-WT SDH-deficient GISTs by examining the genomes, methylomes, gene expression profiles, and *SDHx* mutation status of a cohort of 59 SDH-deficient GISTs patients.

Perhaps the most significant characteristic of SDH-deficient GISTs is that those tumors generally pursue an indolent course despite the high rate of local recurrence and distant metastasis.<sup>[24]</sup> In our study, at the longest follow-up of 34 months, none of the patients died of disease even though five cases (41.7%) presented distant metastasis at or after diagnosis and those underwent disease progression obtained disease control again when the targeted therapy was changed. Similarly, Miettinen et al<sup>[25]</sup> reported that 37 patients in their study had distant metastasis. However, of these patients, only 13 patients (35.1%) died of disease, and there was a favorable median survival of 8.8 years. The remaining 24 patients (64.8%) were alive with metastasis 2 to 43 years after surgery. More strikingly, Mason and Hornick<sup>[15]</sup> reported in their observations that even patients who were classified by conventional criteria as having very low- or low-risk features will often eventually develop metastatic disease. Sixty-seven percent of patients (8/12) with very low-risk and 60% of patients (6/10) with low risk classifications in their study had distant metastasis at a mean follow-up of 8.2 years. Furthermore, in a study of 76 patients regarding the surgical management of WT-GISTs, Weldon et al<sup>[26]</sup> revealed that metastatic disease at diagnosis and mitotic count >5/50 HPFs were prognostic risk factors of event-free survival by multivariate analysis. For those



reasons, the conventional risk stratification based on the modified NIH criteria to predict disease progression might not suitable for patients with SDH-deficient GISTs.

Due to the rarity of SDH-deficient GISTs, the treatment experience of these tumors is relatively limited. Complete surgical removal of the primary tumor and locoregional metastasis is recommended whenever possible, whereas repeated resection after the initial resection significantly decreases postoperative event-free survival.<sup>[26]</sup> In addition, SDH-deficient GISTs generally respond poorly to imatinib due to a lack of activating tyrosine kinase mutations.<sup>[27]</sup> A recent study noted that imatinib directly inhibits metabolic pathways in the submolecular arena of ATP yielding energy producing mitochondrial protein nanomotor functions, where the lack of a cellular ketogenic substrate reserve may explain its failure in SDH-deficient GISTs as well.<sup>[28,29]</sup> As mentioned above, in SDH-deficient GISTs, SDH inactivation leads to the accumulation of HIF-1 $\alpha$ . Meanwhile, HIF, acting as an active transcription factor, induces the expression of downstream genes, including the insulin-like growth factor gene (*IGF*) and vascular endothelial growth factor (*VEGF*). This may explain how sunitinib, a small-molecule that possesses direct cytotoxicity and is an inhibitor of multiple receptor tyrosine kinases, including the *VEGFR* receptor in addition to *PDGFRA* and *KIT* receptors, works well in SDH-deficient GISTs. Interestingly, 4 patients in our study who suffered disease progression during imatinib treatment obtained disease control when the targeted therapy was changed to sunitinib. Furthermore, there is an apparent lack of fumaric acid formation due to SDH deficiency that decreases mitochondrial metabolic matrix water recycling, whereby the natural deuterium depleting capacity of tumor cells from fatty acids upon complete oxidation of the beta carbon are diminished. This is important as the deuterium depleted matrix water yield of mitochondrial ketogenic substrates is strictly dependent on Krebs–Szent–Györgyi cycle functions, where SDH plays a major role. Therefore, it may be clinically important to provide deuterium depleted water for cytoplasmic hydrogen exchange reactions that needs to be incorporated in dieting protocols to achieve more favorable clinical outcomes.<sup>[30–33]</sup> Moreover, IGF and its receptor show potential as therapeutic targets.<sup>[34]</sup>

In conclusion, although we are only able to study 12 cases in our institution, we found that SDH-deficient GISTs comprise a subgroup of a relatively rare tumor type and show a number of clinicopathologic and IHC unique features. The use of IHC to analyze loss of SDHB is reliable for detecting these tumors. SDH-deficient GISTs are restricted to stomach and do not occur infrequently in people older than 40 years. Lymph node metastasis, BVI, local recurrence, and distant metastasis are much more commonly in SDH-deficient GISTs than *KIT/PDGFR*A-mutant GISTs. Nevertheless, they often follow an indolent course despite the high rate of metastasis. Moreover, SDH-deficient GISTs respond poorly to imatinib due to their lack of *KIT/PDGFR*A mutation, whereas sunitinib may work well on account of its ability to inhibit multiply receptor tyrosine kinases.

## References

- [1] Yamamoto H, Oda Y. Gastrointestinal stromal tumor: recent advances in pathology and genetics. *Pathol Int* 2015;65:9–18.
- [2] Doyle LA, Hornick JL. Gastrointestinal stromal tumours: from KIT to succinate dehydrogenase. *Histopathology* 2014;64:53–67.
- [3] Wang YM, Gu ML, Ji F. Succinate dehydrogenase-deficient gastrointestinal stromal tumors. *World J Gastroenterol* 2015;21:2303–14.
- [4] Gill AJ, Chou A, Vilain R, et al. Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. *Am J Surg Pathol* 2010;34:636–44.
- [5] Oudijk L, Gaal J, Korpershoek E, et al. SDHA mutations in adult and pediatric wild-type gastrointestinal stromal tumors. *Mod Pathol* 2013;26:456–63.
- [6] Miettinen M, Lasota J. Succinate dehydrogenase deficient gastrointestinal stromal tumors (GISTs)—a review. *Int J Biochem Cell Biol* 2014;53:514–9.
- [7] Wagner AJ, Remillard SP, Zhang YX, et al. Loss of expression of SDHA predicts SDHA mutations in gastrointestinal stromal tumors. *Mod Pathol* 2013;26:289–94.
- [8] Yang Y, Lane AN, Ricketts CJ, et al. Metabolic reprogramming for producing energy and reducing power in fumarate hydratase null cells from hereditary leiomyomatosis renal cell carcinoma. *PLoS ONE* 2013;8:e72179.
- [9] Janeway KA, Kim SY, Lodish M, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc Natl Acad Sci USA* 2011;108:314–8.
- [10] Mason EF, Hornick JL. Succinate dehydrogenase deficiency is associated with decreased 5-hydroxymethylcytosine production in gastrointestinal stromal tumors: implications for mechanisms of tumorigenesis. *Mod Pathol* 2013;26:1492–7.
- [11] Belinsky MG. Succinate dehydrogenase deficiency in pediatric and adult gastrointestinal stromal tumors. *Front Oncol* 2013;3:117.
- [12] Joensuu H. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol* 2008;39:1411–9.
- [13] Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults. A clinicopathological, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. *Am J Surg Pathol* 2005;29:1373–81.
- [14] Miettinen M, Wang ZF, Sarlomo-Rikala M, et al. Succinate dehydrogenase-deficient GISTs: a clinicopathological, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. *Am J Surg Pathol* 2011;35:1712–21.
- [15] Mason E, Hornick JL. Conventional risk stratification fails to predict progression of succinate dehydrogenase-deficient gastrointestinal stromal tumors. *Am J Surg Pathol* 2016;40:1616–21.
- [16] Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. *J Intern Med* 2009;266:43–52.
- [17] Gaal J, Stratakis CA, Carney JA, et al. SDHB immunohistochemistry: a useful tool in the diagnosis of Carney-Stratakis and Carney triad gastrointestinal stromal tumors. *Mod Pathol* 2011;24:147–51.
- [18] Carney JA. Carney triad. A syndrome featuring paraganglionic, adrenocortical, and possibly other endocrine tumors. *J Clin Endocrinol Metab* 2009;94:3656–62.
- [19] Boikos SA, Pappo AS, Killian JK, et al. Molecular subtypes of KIT/PDGFR A wild-type gastrointestinal stromal tumors: a report from the National Institutes of Health Gastrointestinal Stromal Tumor Clinic. *JAMA Oncol* 2016;2:922–8.
- [20] Yamamoto H, Kojima A, Miyasaka Y, et al. Prognostic impact of blood vessel invasion in gastrointestinal stromal tumor of the stomach. *Hum Pathol* 2010;41:422–1430.
- [21] Corless CL. Gastrointestinal stromal tumors: what do we know now? *Mod Pathol* 2014;27:S1–6.
- [22] Boikos SA, Stratakis CA. The genetic landscape of gastrointestinal stromal tumor lacking KIT and PDGFRA mutations. *Endocrine* 2014;47:401–8.
- [23] Killian JK, Miettinen M, Walker RL, et al. Recurrent epimutation of SDHC in gastrointestinal stromal tumors. *Sci Transl Med* 2014;6:177r–268r.
- [24] Pantaleo MA, Lolli C, Nannini M, et al. Good survival outcome of metastatic SDH-deficient gastrointestinal stromal tumors harboring SDHA mutations. *Genet Med* 2014;17:391–5.
- [25] Miettinen M, Killian JK, Wang ZF, et al. Immunohistochemical loss of succinate dehydrogenase subunit A (SDHA) in gastrointestinal stromal tumors (GISTs) signals SDHA germline mutation. *Am J Surg Pathol* 2013;37:234–40.
- [26] Weldon CB, Madenci AL, Boikos SA, et al. Surgical management of wild-type gastrointestinal stromal tumors: a report from the National Institutes of Health Pediatric and Wildtype GIST Clinic. *J Clin Oncol* 2016;28:JCO2016686733.

- [27] Rege TA, Wagner AJ, Corless CL, et al. “Pediatric type” gastrointestinal stromal tumors in adults: distinctive histology predicts genotype and clinical behavior. *Am J Surg Pathol* 2011;35:494–504.
- [28] Tea I, Martineau E, Antheaume I, et al.  $^{13}\text{C}$  and  $^{15}\text{N}$  natural isotope abundance reflects breast cancer cell metabolism. *Sci Rep* 2016;6:34251.
- [29] Somlyai G, Collins TQ, Meuillet EJ, et al. Structural homologies between phenformin, lipitor and gleevec aim the same metabolic oncotarget in leukemia and melanoma. *Oncotarget* 2017;8:50187–92.
- [30] Lewis CA, Parker SJ, Fiske BP, et al. Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells. *Mol Cell* 2014;55:253–63.
- [31] Somlyai G, Jancsó G, Jákli G, et al. Naturally occurring deuterium is essential for the normal growth rate of cells. *FEBS Lett* 1993;317:1–4.
- [32] Duan JR, Billault I, Mabon F, et al. Natural deuterium distribution in fatty acids isolated from peanut seed oil: a site-specific study by quantitative  $^2\text{H}$  NMR spectroscopy. *Chembiochem* 2002;3:752–9.
- [33] Boros LG, D’Agostino DP, Katz HE, et al. Submolecular regulation of cell transformation by deuterium depleting water exchange reactions in the tricarboxylic acid substrate cycle. *Med Hypotheses* 2016;87:69–74.
- [34] Chou A, Chen J, Clarkson A, et al. Succinate dehydrogenase-deficient GISTs are characterized by IGF1R overexpression. *Mod Pathol* 2012; 25:1307–13.