Original Article
A novel germline mutation in SDHA identified in a rare case of gastrointestinal stromal tumor complicated with renal cell carcinoma

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Abstract: Succinate dehydrogenase (SDH), which is located on the mitochondrial inner membrane, is essential to the Krebs cycle. Mutations of the SDH gene are associated with many tumors, such as renal cell carcinoma, wild type gastrointestinal stromal tumors (WT GISTs) and hereditary paragangliomas/pheochromocytomas. Herein we present a rare case diagnosed as a WT GIST complicated with a renal chromophobe cell tumor and detected a novel germline heterozygous mutation (c.2T>C: p.M1T) in the initiation codon of the SDHA gene. We also conduct a preliminary exploration for the mechanism of reduced expression of SDHB without mutation of SDHB gene. Our case enriches the mutation spectrum of the SDH gene. After reviewing previous studies, we found it to be the first case diagnosed as a WT GIST complicated with a synchronous renal chromophobe cell tumor and identified a novel germline heterozygous mutation. It was also the second reported case of a renal cell carcinoma associated with an SDHA mutation.

Keywords: Succinate dehydrogenase, gastrointestinal stromal tumor, renal cell carcinoma, mutation

Introduction

Succinate dehydrogenase (SDH), also known as complex II, is an essential part of the Krebs cycle. Located on the inner membrane of mitochondria, SDH can not only catalyze conversion of succinate to fumarate, but also plays a role in the electron transport chain. SDH is highly conserved, and consists of four subunits, namely SDHA, SDHB, SDHC and SDHD, whose genes are located respectively on 5q15, 1p36.1-1p35, 1q21 and 11q22.3-23 [1].

Over the last 15 years, many tumor syndromes associated with SDH and accessory factor gene mutations have been identified, which include renal cell carcinomas (RCCs), wild type (WT) gastrointestinal stromal tumors (GISTs) and hereditary paragangliomas/pheochromocytomas. The role that SDH mutations play in tumor genesis has been the subject of intensive research. A variety of hypotheses have been put forward based on the data in hand [1].

RCC has generally been considered a disease resulting from a metabolic disorder, because the relevant genes (such as VHL, MET, and SDH) are involved in fundamental cellular processes regulating the cell response to sensing oxygen, iron, nutrients and energy status [2, 3]. RCC associated with SDH mutation has been reported in patients with paraganglioma/pheochromocytoma syndrome type 4 [4]. Most of them harbor a mutation in the SDHB gene [5-7]. In addition, sporadic cases have also been described with mutations involving the SDHC and SDHD genes [1, 8-10]. Recently, a novel SDHA homozygous deletion in an aggressive variant of RCC was identified by next-generation sequencing, and further study confirmed that the mutation leads to the loss of SDHA and SDHB protein expression [11].

GISTs, first described by Mazur and Clark in 1983, are the most common mesenchymal tumor of the gastrointestinal tract, which is mostly caused by oncogenic mutations in KIT or
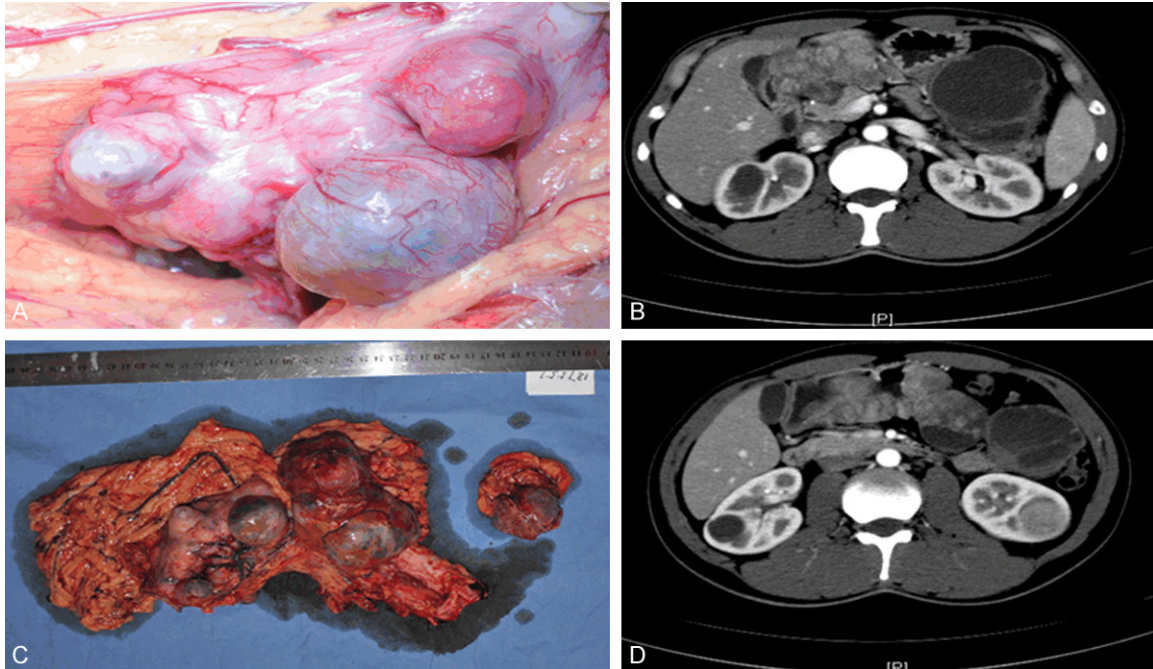
Figure 1. CT and intraoperative findings of the patient. A, C. Laparotomy confirmed multifocal tumors in the gastric wall and discovered a mass in the inferior pole of the left kidney. B, D. Abdominal CT scan identified multifocal gastric tumor and mass occupation on left kidney and right kidney cyst.

PDGFRA. However, about 15% of GISTs do not harbor any mutations in the *KIT* or *PDGRA* genes, and this kind of GIST is called a WT GIST [12, 13]. Most WT GISTs are SDH-deficient and are not driven by *KIT* or *PDGFRA* mutations. SDH-deficient GIST refers to a gastrointestinal stromal tumor with loss of expression of SDH. Among the patients with SDH-deficient GISTs, nearly half harbor *SDH* subunit gene mutations. Of the mutations detected, 60% percent are located on the A subunit and the remaining 40% on B, C or D [14].

Though cases of WT GISTs or RCCs related to *SDH* mutation have both been widely reported, seldom has a patient been diagnosed with both tumors synchronously. We herein report a case of a WT GIST complicated with RCC that had a novel initiation codon germline mutation of the *SDHA* gene, and was also the second reported case of RCC associated with *SDHA* mutation. We also report a preliminary exploration of the typical expression of SDHB and SDHA in the *SDHA* mutant.

Materials and methods

Patient

A 23-year-old man was admitted with a history of hematemesis and melena. He had no fever,

no abdominal pain and no elevated lesions on the skin. He was also thin and pale. Physical examination indicated that a firm, hard mass with a diameter of 6 cm could be palpated in the left upper quadrant. The patient did not present any significant previous medical history, and underwent no regular physical examination. His family members had no similar complains. Routine blood test revealed no remarkable abnormity. Endoscopic examination reported multifocal tumors located in the gastric body and antrum. The largest mass, originating from gastric antrum, was covered by mucosa with an ulcer on the top. Abdominal CT scan demonstrated multifocal gastric tumor, mass occupation on left kidney, and right kidney cyst (**Figure 1B**, **1D**). Even without any family history, retinoscopy and head CT scan were conducted to rule out VHL syndrome. After multidisciplinary team discussion, we decided to resect the tumors of two sites by cooperating with urological surgeons.

A laparotomy on August 27, 2013 confirmed multifocal tumors in the gastric wall; the largest one was located in the gastric antrum. Local lymph nodes were detected without enlargement. Moreover, a tumor with a diameter of 3 cm was discovered in the inferior pole of the left kidney. Finally a total gastric resection and
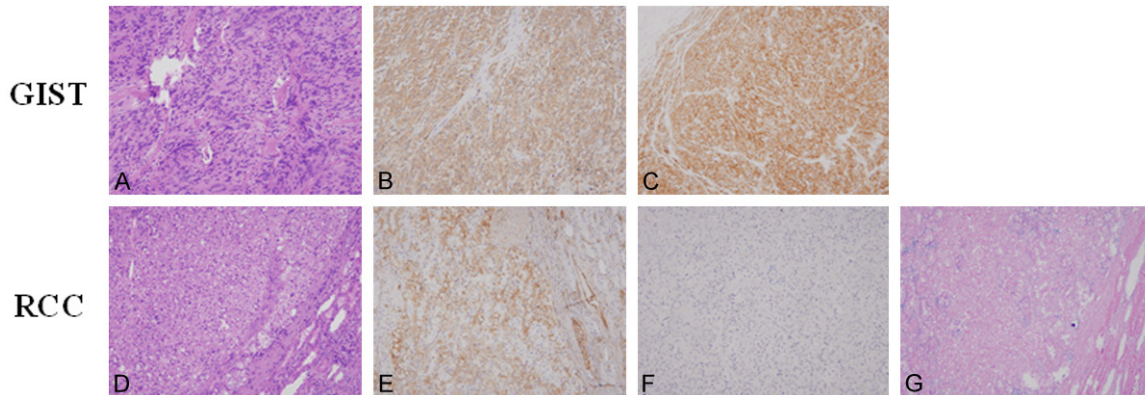
Figure 2. IHC for gastric and renal lesion. (A) HE staining reveals epithelioid cells of full vision. Strong positive expressions of CD 117 (B) and DOG-1 (C) confirm the diagnosis of GISTs. (D) HE staining reveals large and polygonal cells with pale and foamy cytoplasm. IHC reveals that the cell displayed partially positive expression for VIM (E), negative for CK7 (F) and sporadic reticular cytoplasmic positivity for Hale's colloidal iron stain (G).

a partial left kidney resection were performed (**Figure 1A**, **1C**).

A stomach specimen checked by pathologists showed tumors scattered on the gastric wall, of which the largest was 16×8×7 cm. Microscopic analysis revealed epithelioid cells of characteristic shape, intermediate grade atypia and a mitotic count of 3/50 HPF. The tumor had infiltrated the gastric muscular layer. In total, 21 local perigastric lymph nodes were observed with no metastasis. Tumor thrombus was seen in the lymphatic vessels. An immunohistochemistry (IHC) assay revealed that the cell displayed diffuse, strong positive expression of CD117 and DOG-1 (**Figure 2B**, **2C**). The pathology finding indicated a high-risk GIST. The other specimen was derived from the inferior pole of the left kidney, with a size of 4×3.4 cm. Microscopic assay revealed large polygonal cells with pale foamy cytoplasm, an irregular nucleus and clear perinuclear region. IHC revealed that the cell displayed partially positive expression for VIM and negative for CK7. However, Hale's colloidal iron stain showed sporadic reticular cytoplasmic positivity. From these pathological results, the tumor was diagnosed as renal chromophobe cell carcinoma (**Figure 2D-G**).

Considering the presence of CD117 and DOG-1 was strongly positive, the tumors on the gastric walls were confirmed as GISTs. According to our regular process, we run a genetic test for *C-kit* and *PDGFRA* using paraffin-embedded tissue. However, exons [9, 11, 13, 17] of the *C-kit* gene and exons [12, 18] of *PDGFRA* were detected without any mutation. Based on previous stud-

ies, we recognized that *SDH* gene mutation can be found in both WT GISTs and RCCs. Taking the existence of two types of tumor into account, we decided to directly run a test for the *SDH* gene with the consent of patient.

Immunohistochemistry

This study protocol was approved by the Ethical Committee of Zhongshan Hospital. After informed consent, tissue samples and a peripheral blood sample were obtained from the patient. IHC staining for SDH in tissues of the GIST and RCC were performed on 4-µm thick formalin-fixed, paraffin-embedded whole tissue sections with a Dako Autostainer, a polymer-based detection system and a Dako EnVision FLEX High pH kit (Dako, Carpinteria, CA, USA). The following antibodies were used: rabbit monoclonal antibody against SDHC [EPR1103-5(B), Abcam, dilution 1:250], mouse monoclonal antibody against SDHB (clone 21A11AE7, Abcam, dilution 1:400), rabbit monoclonal antibody against SDHD (ab189945, Abcom, dilution 1:250), and mouse monoclonal antibody against SDHA (2E3GC12FB2AE2; Abcom, dilution 1:1000). The results were interpreted as negative when cytoplasmic staining was absent in tumor cells and conversely as positive when cytoplasmic staining was present.

Protein extraction and western blotting for SDHB

Frozen tissues from the patient, including gastric lesions, renal lesions, and normal tissues, were obtained from two sites each. Tissue was
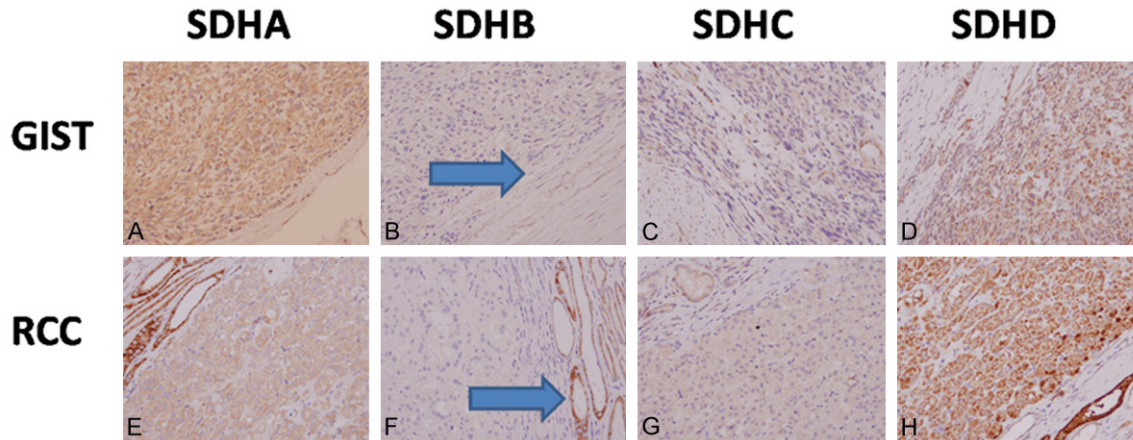
Figure 3. IHC for GISTs and RCC. positive staining for SDHA (A, E), SDHC (C, G), SDHD (D, H) and negative staining for SDHB (B, F). By contrast, normal gastric and renal tissue (arrows) are both SDHB positive.

disrupted in RIPA buffer (Sigma Aldrich St. Louis, MO, USA) mixed with protease inhibitors (1 mM PMSF, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mM orthovanadate sodium salt), and lysed for 1 h with gentle agitation at 4°C. Lysates were centrifuged at 15,000 g for 15 min at 4°C and supernatants were stored at 80°C. Protein concentration was determined with the BCA protein assay (Pierce, Rockford, IL, USA). The samples were named G1and G2 for GIST, R1 and R2 for RCC, and N1 and N2 for normal tissues. After dilution, the concentrations for corresponding samples were 8.8 mg/ml, 4.05 mg/ml, 10.3 mg/ml, 6.1 mg/ml, 8.3 mg/ml, and 5.7 mg/ml, respectively. Proteins were run on a 15% SDS PAGE gel and transferred onto PVDF membrane. Nonspecific binding sites were blocked by incubation in blocking buffer (PBS containing 50 ml 0.1% Tween 20 with 2.5 g skim milk powder) for 1 h at room temperature. Membranes were incubated overnight at 4°C with a mouse monoclonal antibody against SDHB (clone 21A11AE7, Abcam, dilution 1:400) followed by a rabbit polyclonal β-actin antibody (1:500 sc-8432, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Then membranes were washed and incubated with peroxidase conjugated secondary antibodies for 1 h at room temperature. Antigens were revealed using Enhanced Chemiluminescence Reaction (ECL Advance, Amersham Pharmacia Biotech, Les Ulis, France). SDHB expression was quantified by calculating the relative band intensity in grayscale images of the proteins using AlphaView SA software version 3.3.0 (Cell Biosciences Inc.).

Genetic analysis

We also collected samples of tumors and normal tissue. In additions, samples from 100 unrelated population-matched controls were sequenced for mutation to exclude the possibility of a polymorphism in the *SDH* gene. We extracted DNA according to standard methods and designed primers flanking all coding exons and intron-exon boundaries of the *SDH* genes using the web-based version of the Primer 3.0 program (htttp://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). After amplification, the products were purified using a QI-Aquick PCR Purification Kit (Qiagen, Valencia, CA, USA). We sequenced the *SDH* gene using an ABI PRISM 3730 automated sequencer (Applied Biosystems). Sequence comparisons and analysis were performed using the Phred-Phrap-Consed Version 12.0 program. Mutations were identified by comparing the sequence with the reported cDNA reference sequence (GenBank accession number NM_002529).

DNA methylation analysis of the SDHB gene

Based on the results of IHC and genetic tests, we hypothesized that deficiency of SDHB could be attributed to hypermethylation of the *SDHB* gene. To test our hypothesis, DNA was extracted from paraffin-embedded tissues using a DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA was treated with sodium bisulfate using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA). After searching
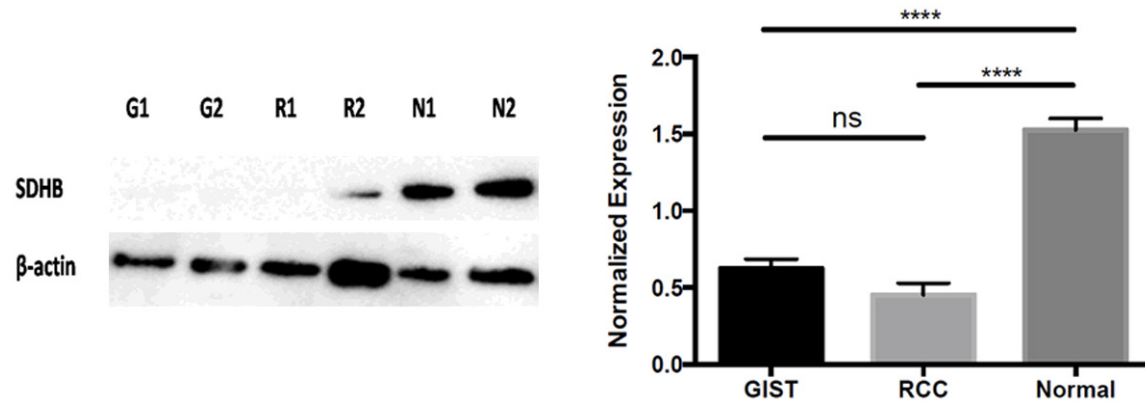
Figure 4. Western blotting for SDHB. G1 and G2 represent GIST derived from different locations of the same tumor, and R1 and R2 represent RCC derived from different locations of the same tumor. N1 is the normal gastric tissue surrounding the GIST. N2 is the normal kidney tissue surrounding the RCC. G1, G2, and R1 were negative. N1 and N2 were c. β-actin for R2 was inconsistent with other samples. Grayscale analysis of the level of SDHB protein in tissues of three origins. SDHB expression markedly increased in GIST and RCC compared with normal tissue; $P<0.001$. SDHB expression was apparently no different between GIST and RCC; *$P>0.05$, **$P<0.1$, ***$P<0.01$, ****$P<0.0001$.

online, the -78~75 region with 17 CpG islands was chosen as the testing target (http://genome.ucsc.edu/cgi-bin/hg). Primers for SDHB were synthesized in our laboratory. The sequence of the forward primer was 5'-GTGGGTT-TTTAGTGGATGTAGGTT-3' and the reverse primer was 5'-TTCCCTCTCTAAAACTCCAAAACT-3'. PCR was carried out with fusion primers with inner template-specific sequences in 30 μl reactions that contained 2 μl bisulfate-converted DNA, 2 μl MgCl$_2$, 3 μl dNTP mixture (10 μM), 1.5 μl forward primer (10 μM) and 1.5 μl reverse primer (10 μM), 16 μl ddH$_2$O and 1 μl TaKaRa LA Taq DNA polymerase (TaKaRa Bio, Osaka, Japan). Amplification of the *SDHB* locus was started by an initial activation of the TaKaRa LA Taq DNA polymerase at 95°C for 15 min. The initial amplification cycle was denaturation at 95°C for 1 min, annealing at 60°C for 2 min, and elongation at 72°C for 3 min. This procedure was continued for 20 cycles, reducing the annealing temperature by 0.5°C each cycle, followed by 40 cycles of a 1 min denaturation at 95°C, 2 min annealing at 50°C, and 2 min elongation at 72°C. The amplification products were collected using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Finally, results were analyzed using a Seqscape v2.7 software package (Applied Biosystems).

Results

The pathology test indicated that our case was a GIST complicated with a renal chromophobe cell tumor. After testing the exons [9, 11, 13, 17] of the *C-kit* gene and exons [12, 18] of *PDGFRA*, no mutation was observed, which convinced us to run an IHC test for SDH. The analysis indicated negative staining for SDHB, partial positive staining for SDHC and positive staining for SDHA and SDHD. These results were consistent for GISTs and RCCs (**Figure 3**).

To confirm the IHC test, western blotting for SDHB was done. The western blot for SDHB indicated negative expression for GIST (G1, G2) and positive for normal tissue (N1, N2). R1 for RCC was negative. However, the β-actin result for R2 was not consistent with the other samples (**Figure 4**). To further confirm our results, we performed a grayscale analysis. These results indicated that the expression of SDHB of both the GIST and RCC was different from the normal tissue (**Figure 4**).

To investigate the cause of defective expression of SDHB, we tested the *SDHB* gene, which was detected without any mutation. After reviewing the earlier studies, we decided to run a test for the *SDHA* gene. The patient was finally identified as having a de novo mutation in *SDHA*. An identical mutation existed in the blood sample, normal tissue and two tumor samples, which indicates a germline heterozy-
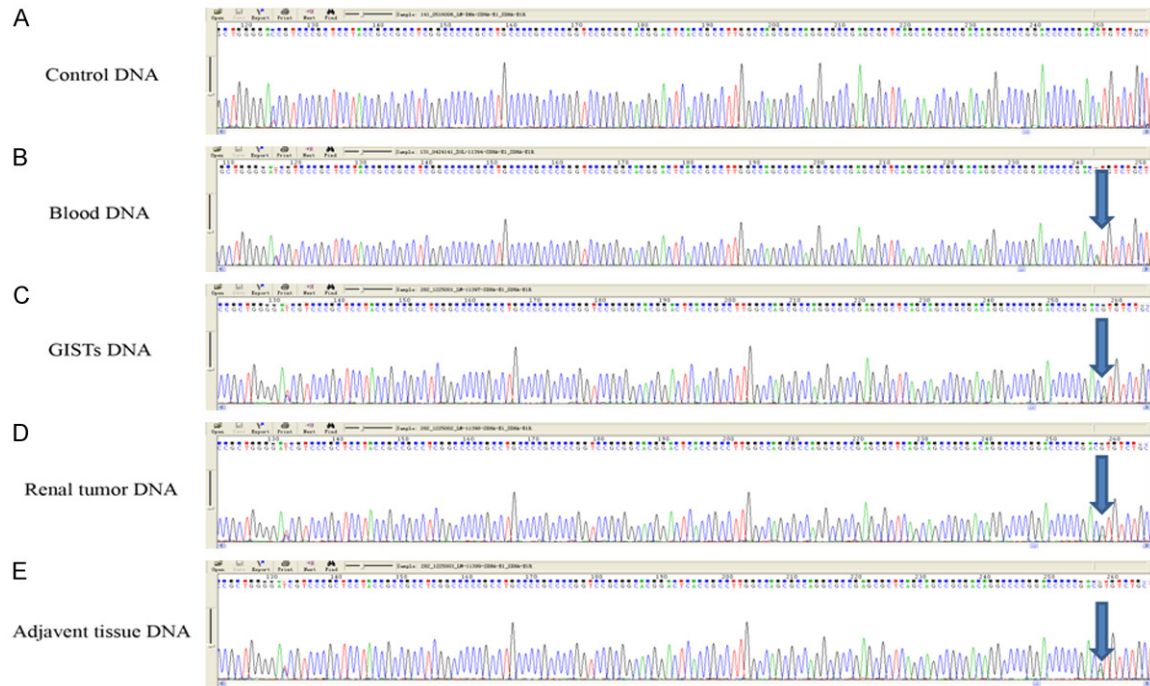
Figure 5. Genetic test for SDHA. The identical mutation existed in blood sample (B), adjacent tissue (E) and two tumor samples (C, D) indicate a germline heterozygous mutation. The mutation (c.2T>C: p.M1T) located in the initiation codon. Control DNA was also tested (A).

gous mutation. The mutation (c.2T>C: p.M1T) was located in the initiation codon (**Figure 5**).

We were confused by the defective expression of SDHB under the condition of a *SDHA* mutation. We suspected that methylation of the *SDHB* gene could be responsible, so we tested the methylation pattern of the *SDHB* gene. Promoter methylation of the *SDHB* gene was infrequent in all tissues, with 1 of 204 CpG sites in 12 clones positive in blood (A), 2 of 187 CpG sites in 11 clones positive in GIST (B), 2 of 170 CpG sites in 10 clones positive in RCC (C) and 4 of 221 CpG sites in 13 clones positive in adjacent tissue (D). These results indicated hypomethylation of the *SDHB* promoter in all tissues (**Figure 6**).

The patient had an uneventful recovery and was finally discharged postoperatively after 2 weeks. After discussion, we recommended that the patient take sunitinib, 25 mg daily, considering that the patient had been synchronously diagnosed with high-risk WT GISTs and renal chromophobe cell carcinoma. No significant adverse reactions were observed. Follow-up showed no evidence of recurrence within 2 years.

Discussion

After a full review of the literature, we found this to be the first case reported of a combined GIST and chromophobe cell carcinoma in the same patient simultaneously. It was also the first pathogenic mutation (at the initiation codon) of *SDHA* that has been detected for either a GIST or RCC.

Cases describing WT GISTs complicated by a RCC heterochronically have been reported. Recently a germline *SDHC* mutation case presenting as recurrent SDH-deficient GIST and RCC was reported [9]. A 59-year-old female who had a SDH-deficient GIST 40 years previously was diagnosed with RCC. The pathology was distinctive and difficult to classify into any subtype. After 2 years, the patient developed liver metastasis [5]. Pandurengan et al. reported 12 GIST patients complicated by RCC, but unfortunately no detailed information about these patients was presented [15]. Compared with all the cases reported before, our case was characterized by an RCC without any symptoms complicated by coexisting SDH-deficient GIST. This prompted us to give such patients a full clinical scan to rule out latent lesions.
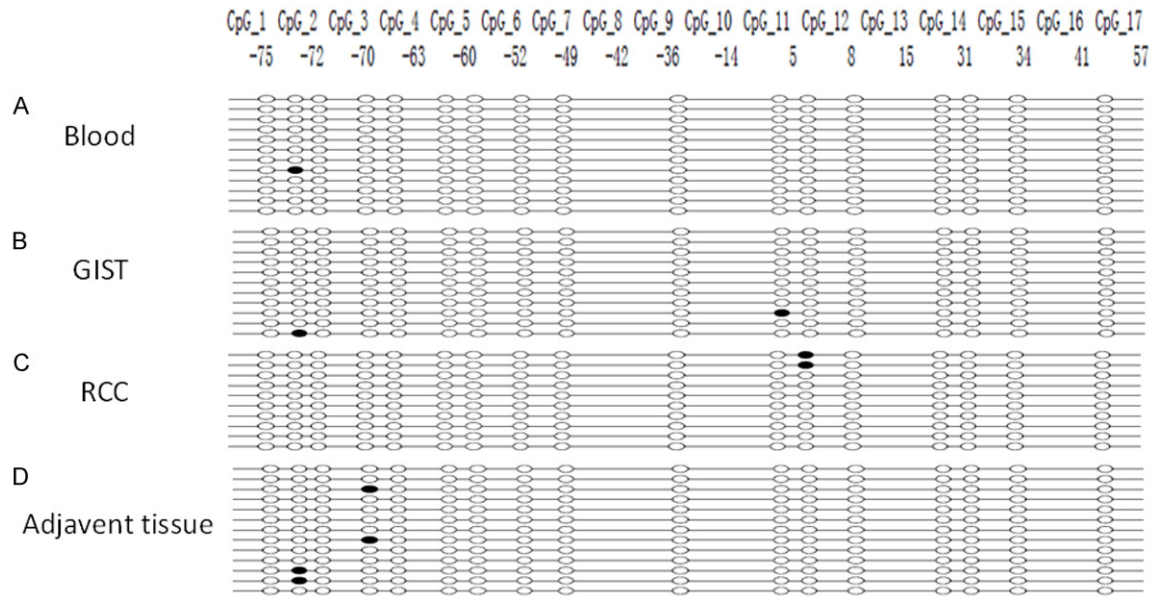
Figure 6. DNA methylation analysis for SDHB (Bisulfite sequencing PCR, BSP). Methylation patterns of individual bisulfate-sequenced clones of the SDHB promoter are shown above. Black and white areas represent, respectively the methylated and unmethylated CpG sites. 1 of 204 CpG sites in 12 clones is positive in blood (A), 2 of 187 CpG sites in 11 clones positive in GIST (B), 2 of 170 CpG sites in 10 clones positive in RCC (C) and 4 of 221 CpG sites in 13 clones positive in adjacent tissue (D).

According to current experience, SDH-deficient GISTs exclusively occur in the stomach. Miettinen et al. reported that this happens at the rate of 7.5% in all gastric GISTs, with no cases found in small intestinal GISTs [16]. Unlike *KIT/PDGFRA* mutation GISTs, SDH-deficient GISTs frequently affect young people. About 90% of pediatric GISTs are SDH-deficient; in the age range of 20-29 years, the rate is 70% percent, and half of gastric GISTs in the age range 30-39 belong to this group [16-18]. In gross morphology, lesions of SDH-deficient GISTs are multifocal and preferentially found in the distal stomach. What surprised us most is that lymphovascular invasion had been observed in 50% of discovered cases. This is extraordinarily rare for such a type of mesenchymal tumor [16]. Compared with other SDH-deficient GISTs, SDHA-negative GISTs are more commonly found in children, with male predominance. In addition, the biological behavior of the tumors was more aggressive, with malignancy more evident in SDHA-negative GISTs, which had a higher rate of liver metastases [16, 19]. Most of the SDH-deficient RCCs reported were eventually identified with an *SDHB* gene mutation. Genotype-phenotype association between *SDHB* alteration and unique tumor morphological characteristics has been

reported [5]. Recently, a case with *SDHA* mutation in renal tumor demonstrated a tumor presenting diverse structures, including papillary, tubulopapillary, cribriform, and collecting duct carcinoma [11]. However, in our case, the tumor of the kidney was diagnosed as chromophobe cell carcinoma based on the results of morphology and IHC. Revealing the relationship between genotype and phenotype of patients with *SDHA* mutations will require more study cases.

As the major catalytic subunit of SDH complex, mutation of the initiation codon of *SDHA* undoubtedly disables part of the function of the whole complex. It has been proved that *SDHA* homozygous deletion can cause the loss of SDHA and SDHB protein detectable by IHC, and loss of expression of SDHA detectable by IHC perfectly matched *SDHA* mutation [11]. However, *SDHA* mutation does not necessarily mean loss of expression of SDHA, which indicates that the function of the other allele is normal. Therefore, SDHA and SDHB have been recommended as markers for screening for potential *SDH* mutations [14, 19]. However, we were surprised to find that IHC for GISTs and chromophobe cell carcinoma were both SDHA positive and SDHB negative in both tumors with identical point mutations. Nevertheless, SDHB stain-

ing for normal tissues around the two tumors is as positive. Our hypothesis was that dysfunction of any component of SDH can lead to instability of the entire complex, resulting in degradation of the SDHB subunit [20]. In order to test this hypothesis, we conducted an IHC test for SDHC and SDHD, for which IHC results have seldom been reported before. According to our hypothesis, the results should both be negative. However, the positive staining observed for both proteins was not consistent with that idea. The results opposite to those expected indicate that the mechanism behind abnormal expression of the SDH subtype needs further study. Though the SDH complex is a vital part of the Krebs cycle and essential for life, haploinsufficiency is tolerated and can be compensated for by a normal allele [14, 20]. In most cases, tumors associated with SDH dysfunction occur via a combination of a loss-of-function germline mutation in one allele and somatic loss-of-function mutations in the tumor cell [16-20]. However, in our case, mutations detected in one allele of both normal and tumor tissue occurred without any mutation in the other allele, indicating that tumors could be found in patients with a germline mutation of one allele.

In order to confirm the IHC results indicating absence of SDHB expression in tumor tissues, we western blotted proteins from frozen tissues. Our results indicated negative expression of G1, G2 and R1, positive for N1 and N2, which is consistent with the IHC results. However, the R2 samples were weakly positive. Since the β-actin result for R2 was not consistent with the other samples. To further confirm our results, we performed a grayscale analysis. These results indicated that the expression of SDHB of both the GIST and RCC was different from the normal tissue.

To further explain the phenomenon of an *SDHA* mutation accompanied by loss of expression of SDHB detectable by IHC without a corresponding *SDHB* mutation, an epigenetic theory has been put forward. Xiao et al. reported that *SDH* knockdown in either a cell or mouse model could elevate intracellular succinate levels and the succinate/a-KG ratio, which would lead to the inhibition of TET enzymes [21]. The function of these enzymes is catalyzing the oxidation of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), which is the first step in the DNA demethylation pathway. That change could lead to hypermethylation in cells [21]. Direct evidence of that theory has been discovered in GISTs. Killian reported that SDH-deficient GISTs present more epigenomic divergence than KIT tyrosine kinase pathway-mutated GIST [22]. It has also been reported that 15 out of 16 patients without mutation of *KIT/PDGFTA/SDH* were detected with hypermethylation in *SDHC*. However, in patients with a germline mutation of *SDHA*, the *SDHC* gene was found to be normal [23]. To explore the mechanism behind the low expression of SDHB in our case, the methylation level of the promoter region of *SDHB* was determined by bisulfate sequencing PCR. A portion of the CpG-rich region around the transcription initiation site of the *SDHB* gene, which spans the 17 CpG island, was sequenced. However, all tissue showed hypomethylation. Still, we could not rule out epigenetic changes as the cause of disease, though aberrant methylation is most frequently seen. More studies are urgently needed.

According to the existing data, WT GISTs are not sensitive to treatment with a tyrosine kinase inhibitor, whether the first-line inhibitor imatinib or the second-line multikinase inhibitor sunitinib; thus, kinase inhibitor treatment may not be prudent for patients that do not harbor a *KIT/PDGFRA* gene mutation [24]. However, WT GISTs, which do not carry any mutation in the pathogenic genes of GISTs, do not respond to drugs targeting them. Since most WT GISTs are SDH-deficient, our team hypothesized that SDH dysfunction may be the cause of drug resistance. After review of the literature, we discovered possible links between SDH dysfunction and drug resistance. When SDH is inhibited in cells, succinate will accumulate, which will lead to a high concentration of succinate in the cytoplasm. A high concentration of succinate inhibits degradation of HIF-1α, which leads to HIF-1α overexpression and HIF translocation into nuclei [25]. The HIF-1 complex can bind to hypoxia response element regions and promote the expression of specific genes, such as NIX and Bnip3, leading to autophagy. It has also been proved that HIF-1α can induce endoplasmic reticulum stress, which will also cause autophagy [26]. Autophagy is involved in the development of imatinib resistance of gastrointestinal stromal tumors [27]. The chain of evidence has made us believe that SDH dysfunc-

tion can play an important role in the development of imatinib resistance for WT GISTs. Relevant studies are now underway in our lab.

In conclusion, our study identified the first case diagnosed simultaneously with GISTs and RCC and also the second RCC accompanied by detection of *SDHA* mutation. Besides, a new pathogenic mutation (initiation codon) of *SDHA* gene is detected, which is reported for the first time for both SDH-deficient GISTs and RCC. Though *SDH* gene mutation has been detected in different tumor, it has still not been proved to be the pathogenic gene. Further study is urgently needed.

Disclosure of conflict of interest

None.

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References

[1] Bardella C, Pollard PJ, Tomlinson I. SDH mutations in cancer. Biochim Biophys Acta 2011; 1807: 1432-1443.

[2] Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 2010; 7: 277-285.

[3] Ricketts CJ, Shuch B, Vocke CD, Metwalli AR, Bratslavsky G, Middelton L, Yang Y, Wei M, Pautler SE, Peterson J, Stolle CA, Zbar B, Merino MJ, Schmidt LS, Pinto PA, Srinivasan R, Pacak K, Linehan WM. Succinate Dehydrogenase Kidney Cancer: An Aggressive Example of the Warburg Effect in Cancer. J Urol 2012; 188: 2063-2071.

[4] Gill AJ, Pachter NS, Clarkson A, Tucker KM, Winship IM, Benn DE, Robinson BG, Clifton-Bligh RJ. Renal tumors and hereditary pheochromocytoma-paraganglioma syndrome type 4. N Engl J Med 2011; 364: 885-886.

[5] Gill AJ, Pachter NS, Chou A, Young B, Clarkson A, Tucker KM, Winship IM, Earls P, Benn DE, Robinson BG, Fleming S, Clifton-Bligh RJ. Renal tumors associated with germline SDHB mutation show distinctive morphology. Am J Surg Pathol 2011; 35: 1578-1585.

[6] Gill AJ, Hes O, Papathomas T, Sedivcova M, Tan PH, Agaimy A, Andresen PA, Kedziora A, Clarkson A, Toon CW, Sioson L, Watson N, Chou A, Paik J, Clifton-Bligh RJ, Robinson BG, Benn DE, Hills K, Maclean F, Niemeijer ND, Vlatkovic L, Hartmann A, Corssmit EP, van Leenders GJ, Przybycin C, McKenney JK, Magi-Galluzzi C, Yilmaz A, Yu D, Nicoll KD, Yong JL, Sibony M, Yakirevich E, Fleming S, Chow CW, Miettinen M, Michal M, Trpkov K. Succinate dehydrogenase (SDH)-deficient renal carcinoma: a morphologically distinct entity: a clinicopathologic series of 36 tumors from 27 patients. Am J Surg Pathol 2014; 38: 1588-1602.

[7] Williamson SR, Eble JN, Amin MB, Gupta NS, Smith SC, Sholl LM, Montironi R, Hirsch MS, Hornick JL. Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. Mod Pathol 2015; 28: 80-94.

[8] Malinoc A, Sullivan M, Wiech T, Kurt WS, Jilg C, Straeter J, Deger S, Hoffmann MM, Bosse A, Rasp G, Eng C, Neumann HP. Biallelic inactivation of the SDHC gene in renal carcinoma associated with paraganglioma syndrome type 3. Endocr Relat Cancer 2012; 19: 283-290.

[9] Gill AJ, Lipton L, Taylor J, Benn DE, Richardson AL, Frydenberg M, Shapiro J, Clifton-Bligh RJ, Chow CW, Bogwitz M. Germline SDHC mutation presenting as recurrent SDH deficient GIST and renal carcinoma. Pathology 2013; 45: 689-691.

[10] Ni Y, Zbuk KM, Sadler T, Patocs A, Lobo G, Edelman E, Platzer P, Orloff MS, Waite KA, Eng C. Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes. Am J Hum Genet 2008; 83: 261-268.

[11] Yakirevich E, Ali SM, Mega A, McMahon C, Brodsky AS, Ross JS, Allen J, Elvin JA, Safran H, Resnick MB. A Novel SDHA-deficient Renal Cell Carcinoma Revealed by Comprehensive Genomic Profiling. Am J Surg Pathol 2015; 39: 858-63.

[12] Agaram NP, Laquaglia MP, Ustun B, Guo T, Wong GC, Socci ND, Maki RG, DeMatteo RP, Besmer P, Antonescu CR. Molecular characterization of pediatric gastrointestinal stromal tumors. Clin Cancer Res 2008; 14: 3204-3215.

[13] Janeway KA, Albritton KH, Van Den Abbeele AD, D'Amato GZ, Pedrazzoli P, Siena S, Picus J, Butrynski JE, Schlemmer M, Heinrich MC, Demetri GD. Sunitinib treatment in pediatric patients with advanced GIST following failure

of imatinib. Pediatr Blood Cancer 2009; 52: 767-771.

[14] Miettinen M, Lasota J. Succinate dehydrogenase deficient gastrointestinal stromal tumors (GISTs)-a review. Int J Biochem Cell Biol 2014; 53: 514-519.

[15] Pandurengan RK, Dumont AG, Araujo DM, Ludwig JA, Ravi V, Patel S, Garber J, Benjamin RS, Strom SS, Trent JC. Survival of patients with multiple primary malignancies: a study of 783 patients with gastrointestinal stromal tumor. Ann Oncol 2010; 21: 2107-2111.

[16] Miettinen M, Wang ZF, Sarlomo-Rikala M, Osuch C, Rutkowski P, Lasota J. Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. Am J Surg Pathol 2011; 35: 1712-1721.

[17] Oudijk L, Gaal J, Korpershoek E, van Nederveen FH, Kelly L, Schiavon G, Verweij J, Mathijssen RH, den Bakker MA, Oldenburg RA, van Loon RL, O'Sullivan MJ, de Krijger RR, Dinjens WN. SDHA mutations in adult and pediatric wild-type gastrointestinal stromal tumors. Mod Pathol 2013; 26: 456-463.

[18] Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, Dahia PL, Liegl B, Ball ER, Raygada M, Lai AH, Kelly L, Hornick JL; NIH Pediatric and Wild-Type GIST Clinic, O'Sullivan M, de Krijger RR, Dinjens WN, Demetri GD, Antonescu CR, Fletcher JA, Helman L, Stratakis CA. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proc Natl Acad Sci U S A 2011; 108: 314-318.

[19] Miettinen M, Killian JK, Wang ZF, Lasota J, Lau C, Jones L, Walker R, Pineda M, Zhu YJ, Kim SY, Helman L, Meltzer P. Immunohistochemical loss of succinate dehydrogenase subunit A (SDHA) in gastrointestinal stromal tumors (GISTs) signals SDHA germline mutation. Am J Surg Pathol 2013; 37: 234-240.

[20] Gill AJ. Succinate dehydrogenase (SDH) and mitochondrial driven neoplasia. Pathology 2012; 44: 285-292.

[21] Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, Liu L, Liu Y, Yang C, Xu Y, Zhao S, Ye D, Xiong Y, Guan KL. Inhibition of alpha-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev 2012; 26: 1326-1338.

[22] Killian JK, Kim SY, Miettinen M, Smith C, Merino M, Tsokos M, Quezado M, Smith WJ, Jahromi MS, Xekouki P, Szarek E, Walker RL, Lasota J, Raffeld M, Klotzle B, Wang Z, Jones L, Zhu Y, Wang Y, Waterfall JJ, O'Sullivan MJ, Bibikova M, Pacak K, Stratakis C, Janeway KA, Schiffman JD, Fan JB, Helman L, Meltzer PS. Succinate dehydrogenase mutation underlies global epigenomic divergence in gastrointestinal stromal tumor. Cancer Discov 2013; 3: 648-657.

[23] Killian JK, Miettinen M, Walker RL, Wang Y, Zhu YJ, Waterfall JJ, Noyes N, Retnakumar P, Yang Z, Smith WJ, Killian MS, Lau CC, Pineda M,Walling J, Stevenson H, Smith C, Wang Z, Lasota J, Kim SY, Boikos SA, Helman LJ, Meltzer PS. Recurrent epimutation of SDHC in gastrointestinal stromal tumors. Sci Transl Med 2014; 6: 177r-268r.

[24] von Mehren M, Randall RL, Benjamin RS, Boles S, Bui MM, Casper ES, Conrad ER, DeLaney TF, Ganjoo KN, George S, Gonzalez RJ, Heslin MJ, Kane JM 3rd, Mayerson J, McGarry SV, Meyer C, O'Donnell RJ, Pappo AS, Paz IB, Pfeifer JD, Riedel RF, Schuetze S, Schupak KD, Schwartz HS, Van Tine BA, Wayne JD, Bergman MA, Sundar H. Gastrointestinal stromal tumors, version 2.2014. J Natl Compr Canc Netw 2014; 12: 853-862.

[25] Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 2005; 7: 77-85.

[26] Wu H, Chen Q. Hypoxia Activation of Mitophagy and Its Role in Disease Pathogenesis. Antioxid Redox Signal 2015; 22: 1032-46.

[27] Rubin BP, Debnath J. Therapeutic implications of autophagy-mediated cell survival in gastrointestinal stromal tumor after treatment with imatinib mesylate. Autophagy 2010; 6: 1190-1191.