The genetic landscape of gastrointestinal stromal tumor lacking *KIT* and *PDGFRA* mutations

Sosipatros A. Boikos · Constantine A. Stratakis

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Abstract About 10–15 % of adult gastrointestinal stromal tumors (GISTs) and 85 % of pediatric GISTs do not have mutations in the *KIT* or *PDGFRA* genes and are generally classified as *KIT*/*PDGFRA* wild type (WT). Recent studies have shown that this group of *KIT*/*PDGFRA* WT GISTs is quite heterogeneous in terms of clinical phenotype, genetic etiology, and molecular pathways. Succinate dehydrogenase subunit (SDH)-deficient GISTs, which include tumors that are part of multiple endocrine neoplasia syndromes, are the newest group of *KIT*/*PDGFRA* WT GIST to be molecularly elucidated. This review aims to describe the different genetic subgroups of *KIT*/*PDGFRA* WT GIST, with a special focus on the SDH-deficient GIST.

Keywords SDH-deficient GIST · BRAF · NF1 · Paraganglioma · Chondroma

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract [1]. GISTs occur with an incidence between 6.8 and 20 per million [2–4]. Activating mutations in the *KIT* or *PDGFRA* genes have been described in GISTs [5, 6] and

several kindreds with familial GISTs due to germline *KIT* or *PDGFRA* mutations have been reported [7–12]. Conventional chemotherapy is ineffective [13], while imatinib and other tyrosine kinase inhibitors (TKI) including sunitinib and regorafenib have shown variable activity in *KIT*/*PDGFRA*-mutated GIST, based on the specific genotype [14–16]. However, imatinib has poor efficacy in *KIT*/*PDGFRA*-“wild type” (WT) GIST. Additional studies are needed to evaluate the efficacy of newer TKIs, such as sunitinib or regorafenib in these tumors [17, 18].

SDH-deficient GIST includes both the syndromic GIST as part of the Carney Triad (CTr) [19, 20] and the Carney–Stratakis Syndrome (CSS) [21], as well as sporadic *KIT*/*PDGFRA* WT GIST [22]. SDH has a central role in energy production, participating in both the Krebs cycle and the respiratory chain. SDH-deficiency leads to accumulation of succinate which subsequently inhibits ten–eleven translocation (TET) and histone lysine (K) demethylases (KDM) enzymes, part of a family of enzymes that are called dioxygenases and participate in the degradation of hypoxia inducible factor 1 alpha (HIF-1a) [23] and the demethylation of DNA and histones [24]. Hydroxylation of 5-methylcytosine (5mC) groups and generation of 5-hydroxymethylcytosine (5hmc) were only recently described [25]. It has been shown that SDH-deficient GIST is characterized by a significant deficiency of 5hmc, consistent with a failure in TET2 maintenance demethylation [24]. The inhibition of this process by succinate accumulation is another example of how Krebs cycle dysfunction may lead to oncometabolite accumulation and epigenetic effects [26–28] (Fig. 1).

BRAF mutations have also been described in *KIT*/*PDGFRA* WT GIST [29]. It is also known that patients with neurofibromatosis have frequently multifocal GIST [30]. This review aims to provide the current data for the clinical and genetic heterogeneity of *KIT*/*PDGFRA* WT

S. A. Boikos (✉)
The Sidney Kimmel Comprehensive Cancer Center at Johns
Hopkins, Baltimore, MD, USA
e-mail: sboikos1@jhmi.edu

C. A. Stratakis
Eunice Kennedy Shriver National Institute of Child Health and
Human Development, Bethesda, MD, USA

Fig. 1 Succinate dehydrogenase (complex II) and epigenetic regulation. *TET* ten–eleven translocation, *KDM* lysine (K) demethylase, *PHD* prolyl hydroxylase

Fig. 2 A classification of gastrointestinal stromal tumors

GISTs. We focus in the SDH-deficient GISTs, which include syndromic (CTr, CSS) and not syndromic GISTs (Fig. 2).

SDH-deficient GISTs

Carney triad

In 1977, Carney described the association of gastric leiomyosarcomas (now known to represent GISTs) with functional paragangliomas and pulmonary chondromas (PCH) [19]. This disorder affected primarily young women. The multifocality in multiple organs and the young age of the patients suggested a genetic disorder. This association was later referred as CTr [31]. Later, patients with CTr were found to have additional tumors such as esophageal leiomyoma and adrenal cortical adenoma [32].

In 1999, Carney reported 79 cases of the triad. Only one-fifth of the patients had all three tumors, and because of the rarity of the disease, the presence of any two of the components was sufficient for the diagnosis. Two of the 79 patients, each with two elements of the triad (gastric sarcoma and paraganglioma) had a sibling with a paraganglioma, raising the possibility that the dyad of gastric sarcoma (GISTs) and paraganglioma constitute a familial syndrome, distinct from CTr [20].

CTr remains until today without clear etiology. Comparative genomic hybridization in 37 patients with Carney Triad has shown genetic alterations in the 1q, 1p, and less frequently in the 14q and 22q chromosomal loci [33]. While *SDHB* is located on chromosome 1p36.1–p35 and *SDHC* in 1q21–q23.3, sequencing for *SDHB*, *-C* and *-D* genes did not identify any mutations in the majority of patients with CTr (8).

CTr GISTs have negative staining for SDHB by immunohistochemistry (ICH) regardless of the absence of

SDHx mutations [34]. GISTs in CTr patients compared with *KIT*- or *PDGFRA*-mutated GIST has unique features: predominance in young women, epithelioid histology, gastric location, multifocality, and frequent lymph node metastases [35].

Carney–Stratakis syndrome

CSS was firstly described in 2002 as a separate condition, characterized by the development of gastric GISTs and paragangliomas, and transmitted by autosomal dominant inheritance with incomplete penetrance [20]. Later, inactivating mutations in *-C* or *-D* genes were found in CSS patients [21, 36]. Loss of heterozygosity around the *SDH* gene chromosomal loci suggests a tumor-suppressor function of SDH subunits in this disease [36].

GISTs in CSS patients has also negative staining for SDHB by ICH [34]. CSS patients present with an almost 1:1 female: male ratio, older age than CTr patients, but do share with CTr patients the epithelioid histology, primarily gastric location, multifocal character, and frequent lymph node metastases [35].

Sporadic SDH-deficient GIST

Frequently, patients with *KIT/PDGFRA* WT GIST and no personal or family history of other tumors like paragangliomas or chondromas have SDHB deficiency by IHC, with or without *SDHx* mutations. In a recent report, four of 34 patients (12 %) with sporadic *KIT/PDGFRA* WT GIST were found to have mutations in *SDHB* or *SDHC* and subsequent SDHB deficiency by IHC [22], while patients can have also isolated SDHB-deficient GISTs without *SDHx* mutations. It is unclear if these isolated SDH-deficient GISTs are sporadic GISTs or the first presentation of CTr or CSS. These patients have to be monitored frequently for symptoms of paragangliomas. Sporadic SDH-deficient GIST shares some of the characteristics of GIST in CTr and/or CSS patients: female predominance (in CTr), gastric location, multifocality, epithelioid histology, and metastasis to regional lymph nodes.

Recent data demonstrate that *SDHA* is the most commonly mutated *SDH* subunit in sporadic WT GIST, with approximately one-fourth of SDHB-deficient GISTs by IHC to harbor *SDHA* mutations. Only one family, including two patients with *SDHA*-mutated GIST has been reported so far [37]. In one study, nine out of 33 (27 %) of SDH-deficient GISTs lacked expression of SDHA; nonsense or missense *SDHA* mutations were found in all these patients [38]. In another study, 36 out of 127 (28 %) SDH-deficient GISTs had loss of SDHA expression. Compared with patients with GISTs with positive staining by IHC, the patients with SDHA-deficient GISTs had a lower female-to-male ratio and an older median age [39].

Non-SDH-deficient GIST

NF1-associated GIST

Von Recklinghausen disease, is an inherited, autosomal dominant disease due to mutations to *NF1* gene, phenotypically characterized by multiple café au lait spots, freckling, Lisch nodules, development of neurofibromas, and in some case includes also other malignancies like brain tumors, leukemia, and GIST. GISTs in NF1 patients are located in the small intestine (including the duodenum), have spindle histology, are multifocal, lack somatic *KIT* and *PDGFRA* mutations, and ICH for SDHB is positive [40–42]. *NF1*-mutated GISTs do not overexpress IGF1R [43]. There is no clear female predominance in this subgroup of *KIT/PDGFRA* WT GIST. There is little data regarding response of *NF1*-mutated GIST to imatinib and sunitinib. One patient has been reported with initial response to imatinib, who eventually became resistant and progressed [44]. In another study, there was no response of *NF1*-mutated GISTs to Imatinib [45].

GIST with *BRAF* mutations

BRAF belongs to the RAF family of serine/threonine protein kinases participating in the RAS-RAF-ERK pathway that regulates cell cycle through activation of MAPK pathway. Mutations in *BRAF* have been identified in other cancers like melanoma and thyroid cancer. In a study that was screening imatinib-naïve and imatinib-resistant GISTs, a *BRAF* exon 15 V600E mutation was identified in three of 61 imatinib-naïve GIST patients. One identical V600E *BRAF* mutation was identified in one of 28 imatinib-resistant GISTs. The tumors were located primarily at the small bowel, were strongly KIT immunoreactive with high mitotic rate, and had a high risk for metastases [46]. In another, more recent study, *BRAF* exon 15 V600E mutation was detected in 9 (13 %) of 70 patients with *KIT/PDGFRA* WT GIST [29].

Mutations in *BRAF* have been described also as a mechanism of resistance. In naïve GISTs carrying activating mutations in *KIT* or *PDGFRA* a concomitant activating mutation in *BRAF* (about 2 %) genes was consistent with resistance. In this study, in vitro experiments showed that imatinib was able to switch off the mutated receptor *KIT* but not the downstream signaling triggered by the *BRAF* mutation [47]. There are no sufficient data of efficacy of imatinib in *BRAF*-mutated GISTs. There is one case of response of *BRAF*-mutated GIST to dabrafenib, a potent ATP-competitive inhibitor of BRAF. In this study, the patient treated with dabrafenib had prolonged antitumor activity [48].

Pathogenesis, clinical implications, and treatment choices in *KIT/PDGFRA* WT-GISTs

GISTs are hypothesized to originate from the interstitial cells of Cajal (or a partially committed precursor cell) [49], the pacemaker cells which regulate peristalsis at the gastrointestinal tract. Since GISTs originate from Cajal cells, they share also immunohistochemical staining for KIT. Other common markers in GISTs are CD34 (hematopoietic progenitor cell antigen) and DOG-1 (ANO1, anoctamin 1) [50]. As we have already mentioned, GISTs in patients with CTr, CSS, and occasionally sporadic GISTs have SDHB deficiency by IHC [34], and *NF1*-mutated GISTs have normal SDHB staining [42], while there are no data yet for SDHB staining in *BRAF*-mutated tumors. GISTs with *SDHA* mutations also have negative SDHB IHC but also do not stain positive for SDHA [22, 51]. SDHB-deficient GIST tends to have epithelioid cytology, lymphovascular invasion, and a characteristic multinodular or plexiform growth pattern [52, 53]. Cells of GISTs with *BRAF* and *NF1* mutations have primarily spindle-like histology.

The exact mechanism of SDH deficiency without *SDHx* mutations is still unclear. One possible mechanism for the observed loss of SDHB expression and complex II function in the *KIT/PDGFRA* WT GISTs samples without any *SDHx* defects is epigenetic modification resulting in decreased mRNA expression of one of the subunits of the SDH complex. Although mRNA expression of *SDHB*, *SDHC*, and *SDHD* has not been found significantly different between *KIT/PDGFRA* WT and mutant GISTs [22], larger studies are needed to confirm this. Another potential mechanism would be mutations in *SDHAF2*, which has been described previously in paragangliomas [54]; *SDHAF2* mutation analysis, which has been conducted in 42 of the *KIT/PDGFRA* WT GIST cases previously, has not revealed any mutations [22]. It is possible that SDHB deficiency in patients with CTr is due to alterations of genes that regulate SDH stability and function.

As it was discussed previously, SDH deficiency is leading to succinate accumulation, which inhibits the demethylation process of DNA and histones. SDH-deficient GIST has been shown to have a hypermethylator phenotype versus *KIT* mutant GIST [24] (Fig. 1). In the same study, comparison of *SDH*-mutant GIST with isocitrate dehydrogenase-mutant glioma revealed comparable measures of global hypo- and hypermethylation. In another study in paragangliomas, *SDHx*-mutated tumors demonstrated a similar hypermethylator phenotype, associated with downregulation of key genes involved in neuroendocrine differentiation [55]. *SDHx* mutations have also been found to predispose to pituitary and renal cell carcinomas [56–58].

While it is known that SDH deficiency is leading to a hypermethylator phenotype, it is unknown how epigenetic dysfunctions can drive tumorigenesis in these tumors. Although KIT is overexpressed in *KIT/PDGFRA* WT GIST, it is unclear if it plays an oncogenic role or is overexpressed because of origin from the interstitial cells of Cajal. In a recent analysis of miRNAs in GIST, a cluster of miRNAs, located in 14q32, was found to be downregulated [59]. Among those, miR-494 have been found in a previous study to downregulate KIT in GIST882 cells [60]. Other confirmed targets by miRNAs located in 14q32 are BCL-6 (miR-127), CCND2 (miR-154), and HDAC6 (miR-433) [59].

Although there is sufficient evidence that the response of SDH-deficient GIST to imatinib is poor, more studies are needed to evaluate the response to other TKIs-like sunitinib that targets angiogenesis, or regorafenib. Patients with SDH-deficient GIST had a median progression-free survival of 12 months [61], while another patient with SDH-deficient GIST had a prolonged response to pazopanib [62]. Another treatment approach would be to target the IGF1R pathway. IGF1R overexpression in *KIT/PDGFRA* WT GIST has been reported in several studies. Indeed, IGF1R is expressed and activated in all GISTs but is markedly overexpressed in *KIT/PDGFRA* WT GISTs compared with mutant *KIT/PDGFRA* GISTs [43, 63, 64]. In a recent study, in a set of 11 patients with *KIT/PDGFRA* WT GIST, eight tumors were SDHB-negative by IHC and showed IGF1R overexpression, while three *NF1*-related tumors were SDHB-positive and IGF1R-negative, suggesting that IGF1R overexpression could be a feature of SDHB-deficient GIST [65]. Results in an ongoing study using linsitinib, an IGF1R inhibitor, in patients with WT GIST (NCT01560260) are currently pending.

There is currently another ongoing trial using vandetanib in *KIT/PDGFRA* WT GIST patients (NCT02015065). Vandetanib targets RET oncogene and is approved for medullary thyroid carcinoma [66, 67]. It has been shown that MTC patients have increased amino-acid coding polymorphisms in *SDHB* as well as G12S and H50R changes in *SDHD*, especially patients with familial tumors, suggesting a functional connection of coding *SDHx* polymorphisms to activating Ret mutations [68]. Finally, since tumors with SDH deficiency seem always to be related with a hypermethylator phenotype, epigenetic modulators like decitabine, either alone or in combination with TKIs could be potentially a reasonable therapeutic approach in *KIT/PDGFRA* WT GIST patients. This is supported by recent preclinical studies, where the migratory phenotype established by hypermethylation in SDHB-deficient chromaffin cells was reversed by decitabine treatment [55].

SDH-deficient GIST has unique characteristics with important implications in terms of screening, natural

history, follow-up, and treatment. Although SDH-deficient GIST has the tendency to metastasize early to liver and lymph nodes, patients frequently have an indolent course, surviving for decades. Risk stratification, by tumor size and mitotic index, using the Miettinen system is not applicable to these tumors [50]. Once a germline *SDHx* mutation is found in a patient with SDH-deficient GIST, it is very important to screen other members of the family for the same mutation. There are no established guidelines yet for follow-up for asymptomatic carriers of *SDHx* mutations. In a recent study, 37 asymptomatic *SDHx* carriers were screened with rapid sequence whole-body MRI. Among those, five patients were diagnosed with six tumors (paragangliomas and one renal cell carcinomas), demonstrating the significance for screening in *SDHx* carriers [69]. Biochemical screening with yearly measurements of plasma catecholamines should be performed also in these patients. Finally, since patients with SDH-deficient GIST may develop GIST or paraganglioma after many years, a long-term clinical follow-up for the identification of GIST or paragangliomas is essential.

Summary

Both CSS and CTr are linked to SDH deficiency either due to *SDHx* mutations or due to unknown factors that lead to SDH deficiency. Patients with either “sporadic” GISTs or paragangliomas should always be checked for both tumors, since they may develop these tumors, years later as part of CSS or CTr. The common SDH deficiency leading to a hypermethylator phenotype in CTr, CSS, and the SDH-deficient sporadic GIST raise the need for the use of the term “SDH-deficient GIST,” which is more specific than *KIT/PDGFR* WT GIST.

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