

Succinate dehydrogenase-deficient gastrointestinal stromal tumors

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represent specific examples of SDH-deficient GISTs. SDH-deficient GISTs locate exclusively in the stomach, showing predilection for children and young adults with female preponderance. The tumor generally pursues an indolent course and exhibits primary resistance to imatinib therapy in most cases. Loss of succinate dehydrogenase subunit B expression and overexpression of insulin-like growth factor 1 receptor (IGF1R) are common features of SDH-deficient GISTs. In WT GISTs without succinate dehydrogenase activity, upregulation of hypoxia-inducible factor 1 α may lead to increased growth signaling through IGF1R and vascular endothelial growth factor receptor (VEGFR). As a result, IGF1R and VEGFR are promising to be the novel therapeutic targets of GISTs. This review will update the current knowledge on characteristics of SDH-deficient GISTs and further discuss the possible mechanisms of tumorigenesis and clinical management of SDH-deficient GISTs.

Key words: Gastrointestinal stromal tumors; Succinate dehydrogenase; Insulin-like growth factor 1 receptor; Vascular endothelial growth factor receptor; Hypoxia-inducible factor 1 α

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Abstract

Most gastrointestinal stromal tumors (GISTs) are characterized by KIT or platelet-derived growth factor alpha (PDGFRA) activating mutations. However, there are still 10%-15% of GISTs lacking KIT and PDGFRA mutations, called wild-type GISTs (WT GISTs). Among these so-called WT GISTs, a small subset is associated with succinate dehydrogenase (SDH) deficiency, known as SDH-deficient GISTs. In addition, GISTs that occur in Carney triad and Carney-Stratakis syndrome

Core tip: Succinate dehydrogenase (SDH) deficiency occurs in about 5%-7.5% of gastrointestinal stromal tumors (GISTs). These so-called SDH-deficient GISTs lack KIT and PDGFRA mutations. Such type of GISTs has its own clinical, morphological and molecular characteristics. The accumulation of hypoxia-inducible factor 1 α and the upregulation of its downstream molecules, such as insulin-like growth factor 1 and vascular endothelial growth factor receptor, may play important roles in the tumorigenesis of SDH-deficient GISTs. They are promising to be the novel therapeutic targets of GISTs.

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. They are most common in the stomach (50%-60%) and small intestine (30%-35%) and are less frequent in the colon and rectum (5%) and the esophagus (< 1%)^[1]. Furthermore, GISTs are able to grow within the abdominal cavity, usually in the omentum, mesentery or the retroperitoneum (< 5% of all GISTs)^[2]. Most GISTs are driven by gain-of-function mutations in *KIT* (75%-80%) or *PDGFRA* (10%)^[3-6]. In addition, 10%-15% of GISTs do not have detectable *KIT* or *PDGFRA* mutations and are called *KIT*/*PDGFRA* wild-type GISTs. It has been reported that succinate dehydrogenase subunit (*SDHA*, *SDHB*, *SDHC* and *SDHD*) mutations, neurofibromatosis 1 (*NF1*) gene mutations, retrovirus-associated DNA sequences (RAS) family mutations (*HRAS*, *NRAS*, *KRAS*) and *BRAF* mutations may contribute to these so-called "wild-type" tumors^[7-10]. Among these, SDH-deficient GISTs account for between 5% and 7.5% of all unselected apparently sporadic gastric GISTs, including the majority of pediatric GISTs (<18 years old) and a small proportion of young adult GISTs (18-30 years old)^[11,12]. The GISTs that occur in Carney triad (the non-familial association of gastric GISTs, pulmonary chondroma and extra-adrenal paraganglioma) and Carney-Stratakis syndrome (the familial association of GISTs and paraganglioma) represent specific examples of SDH-deficient GISTs^[11-15].

Succinate dehydrogenase (succinate-coenzyme Q reductase or mitochondrial complex II) consists of four subunit proteins (*SDHA*, *SDHB*, *SDHC* and *SDHD*) and two succinate dehydrogenase assembly factors (*SDHAF1* and *SDHAF2*)^[16]. *SDHA* and *SDHB* comprise the catalytic component of the succinate-coenzyme Q reductase, whereas *SDHC* and *SDHD* comprise the anchoring component which attaches the complex to the inner mitochondrial membrane. *SDHAF1* may be associated with *SDHB* and may be involved in the insertion or retention of the complex II Fe-S center; *SDHAF2* is required for insertion of the FAD co-factor into *SDHA*^[16,17]. The SDH localized in the inner mitochondrial membrane plays an integral role in cellular metabolism. This complex acts at the interphase of the tricarboxylic acid cycle and electron transport chain and catalyzes the oxidation of succinate to fumarate^[18,19]. Deficiencies of complex II are responsible for many diseases, including hereditary paragangliomas and pheochromocytomas, a specific

group of renal cell carcinomas, a subset of Leigh syndrome, infantile leukoencephalopathy and a certain type of GISTs. In 2006, one study reported a family with multiple paragangliomas (including a patient with GIST) that carried a germline *SDHB* mutation; the authors proposed the relationship between GISTs and SDH mutations for the first time^[20]. A year later, McWhinney *et al.*^[21] identified six germline *SDHB*, *SDHC*, and *SDHD* mutations in GIST patients with the Carney-Stratakis syndrome. Then, numerous studies confirmed the *SDHA*, *SDHB*, *SDHC*, and *SDHD* mutations in GIST patients^[22-26].

The insulin-like growth factor (IGF) system, which is composed of IGF ligands (IGF1 and IGF2), receptors (IGFR and the insulin receptor) and six regulatory IGF-binding proteins (IGFBP1-6), plays a critical role in the growth and development of many tissues and regulates overall cell growth^[27]. The IGF1 receptor (IGF1R) is a transmembrane receptor that interacts with both IGF1 and IGF2, and has been proven to be necessary for transforming several oncogenes^[28-30]. It has also been reported that IGF1 has the potential to stimulate the proliferation of tumor cells *in vitro*^[31]. Genetic manipulations that reduced IGF signaling, such as by *IGF1* gene knockout or by growth hormone (GH) antagonist transgene, can lead to decreased tumor growth in mouse models^[32,33]. In addition, epidemiological evidence indicates that the IGF1 levels influence cancer risk and/or cancer prognosis^[34,35]. We will now summarize the clinical traits and biological events contributing to the tumorigenesis of SDH-deficient GISTs and the rationale for antineoplastic agents.

CLINICAL AND MORPHOLOGICAL FEATURES OF SDH-DEFICIENT GISTs

SDH-deficient GISTs account for 5% to 7.5% of all unselected GISTs^[11,12]. These tumors have a tendency to appear in children and young adults. Nearly all gastric GISTs patients < 18 years old and a substantial percentage of patients < 30 years old belonged to this group. However, the older adult patients rarely displayed an SDH-deficient pattern. Additionally, there was a female predominance greater than 2:1, although the gender distribution was equal in age groups > 30 years^[36].

SDH-deficient GISTs occur exclusively in the stomach, and although any part of the stomach can be involved, there is some predilection to the distal stomach and antrum. Common clinical manifestations of SDH-deficient GISTs are similar to other gastric tumors, including gastrointestinal bleeding and epigastric discomfort. Occasional patients who were diagnosed with SDH-deficient GISTs showed symptoms related to metastatic tumors in the abdomen or liver^[37]. In general, these tumors pursue an indolent course and may sometimes be fatal. Given their lack of oncogenic

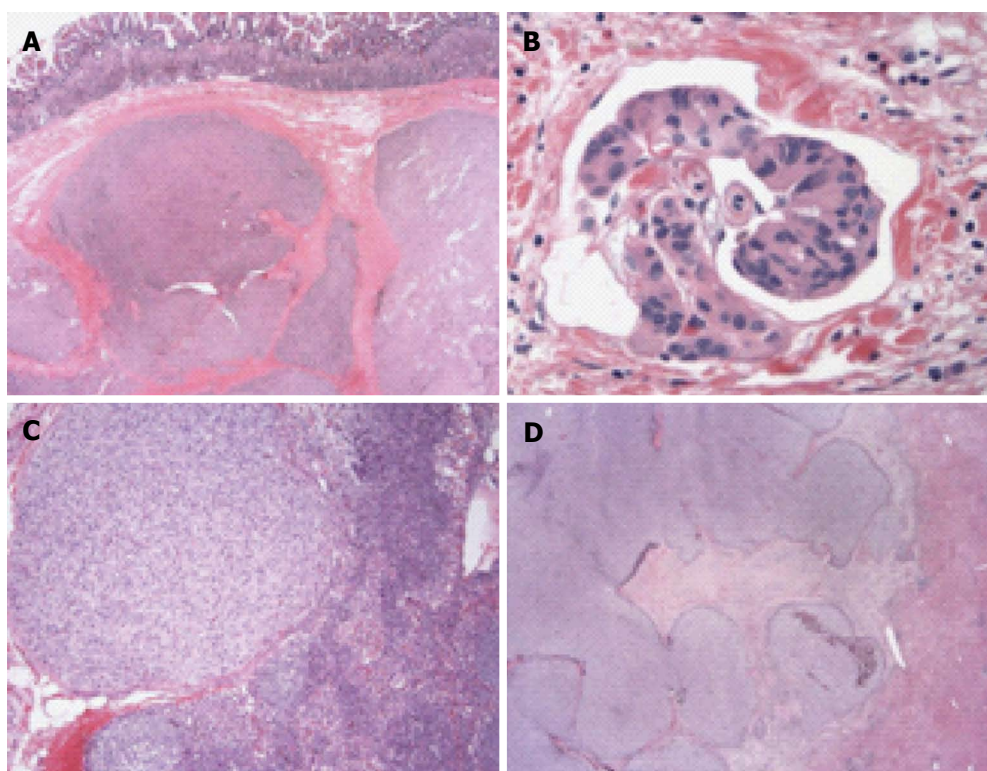


Figure 1 Histological features of succinate dehydrogenase-deficient gastrointestinal stromal tumors. A: Multinodular architecture; B: Lymphovascular invasion; C: Lymph node metastasis; D: Liver metastasis with a multinodular/lobulated architecture similar to that seen in gastric primary SDH-deficient tumors. Data adapted from Barletta *et al*^[50].

activating tyrosine kinase mutations, SDH-deficient GISTs show consistent primary resistance to imatinib therapy^[11-14,38,39].

Carney's triad, which is commonly found in girls and young women, is a rare non-heritable syndrome consisting of gastric GIST, paraganglioma, and pulmonary chondroma^[39-41]. *KIT* or *PDGFRA* mutations were undetectable in GISTs associated with Carney's triad, although the loss of expression of *SDHB* can be observed within the tumors^[14,42]. Carney-Stratakis syndrome is characterized by the development of gastric GISTs and paragangliomas. Unlike Carney's triad, it is inherited in an autosomal dominant manner and affects both men and women^[43]. These tumors also lack *KIT* and *PDGFRA* mutations and have been proven to be SDH-deficient, according to immunohistochemical staining results, which indicates defects in *SDHB*^[13,22]. Moreover, in a series of 11 patients with Carney-Stratakis syndrome, eight were found to harbor germline mutations in *SDH* genes (5 *SDHB*, 2 *SDHC*, and 1 *SDHD*)^[22], whereas GISTs of Carney triad do not harbor *SDHA*, *SDHB*, *SDHC*, or *SDHD* mutations^[42,44]. Pediatric GISTs, which account for 1% to 2% of GISTs, often occur in young girls. The majority of pediatric GISTs, despite showing strong *KIT* immunoreactivity, lack *KIT* and *PDGFRA* mutations and display a characteristic similar to GISTs associated with Carney triad and Carney-Stratakis syndrome patients^[45]. Recently, it was recognized that occasional

GISTs in adults, so-called "pediatric-type" GISTs, show virtually identical features to those observed in pediatric patients^[38,46]. Thus, the clinical features of Carney triad, Carney-Stratakis syndrome, pediatric GISTs, and adult pediatric-type GISTs are strikingly similar. Therefore, we can reason that these GISTs are similar because they are all SDH-deficient.

As for morphological traits, SDH-deficient GISTs are typically described as multinodular or, sometimes, bilobed masses, often divided by apparent fibrous septa. Ulceration was frequently found in such GISTs. Multifocal disease, lymphovascular invasion around the tumor nodules and lymph node metastases are also common, whereas these features are extraordinarily rare in conventional *KIT*-mutant GISTs (Figure 1). In contrast to pure spindle cells in adult mutant GISTs, SDH-deficient GISTs are often composed of epithelioid or mixed epithelioid and spindled cells^[12,17,25,47,48] (Figure 2).

MOLECULAR CHARACTERISTICS OF SDH-DEFICIENT GISTS

Loss of *SDHB* expression is a consistent feature of SDH-deficient GISTs, whereas *SDHB* expression is intact in *KIT*-mutant GISTs^[11,12,49]. As mentioned above, most pediatric GISTs, as well as a small proportion of young adult GISTs, and GISTs in patients with Carney triad or Carney-Stratakis syndrome, have been found to

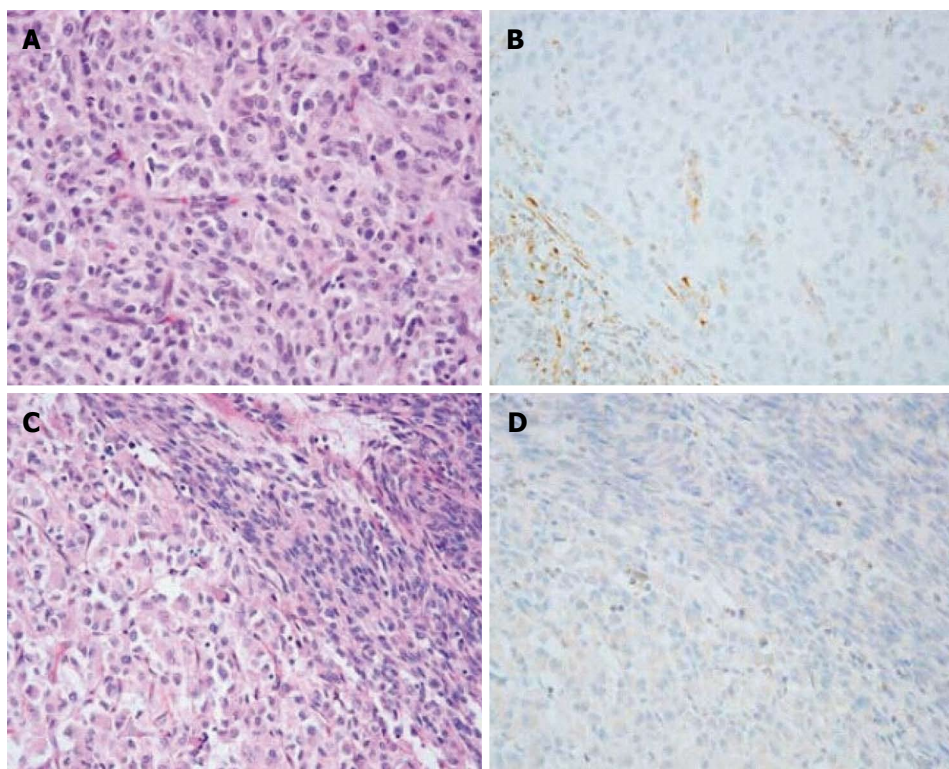


Figure 2 Morphological features of succinate dehydrogenase-deficient gastrointestinal stromal tumor cells. A: Epithelioid gastrointestinal stromal tumor (GIST) cells (HE staining); B: Loss of expression of succinate dehydrogenase subunit B (SDHB, immunohistochemistry); C: Mixed epithelioid and spindle GIST cells (HE staining); D: Loss of expression of SDHB (immunohistochemistry). Data adapted from Wagner *et al*.^[25]

be SDHB deficient^[11,13,14]. Succinate dehydrogenase consists of four subunit proteins (SDHA, SDHB, SDHC and SDHD). Lack of any component of the mitochondrial complex II will result in the instability of the entire complex and the degradation of the SDHB subunit. Therefore, immunohistochemistry for SDHB becomes negative whenever there is a mutation/inactivation of SDHA, SDHB, SDHC or SDHD; negative staining for SDHB is now validated as a highly sensitive marker for germline mutations of any of the SDH subunits^[11,14,48,50,51].

In 2012, Chou *et al*.^[52] proposed that IGF1R overexpression is a feature of SDH-deficient GISTs. They assessed SDHB and IGF1R expression by immunohistochemistry in eight confirmed SDH-deficient GISTs, three GISTs arising in the setting of neurofibromatosis type 1 syndrome and 40 unselected GISTs. Selected *KIT* and *PDGFRA* exons were amplified and sequenced. All eight SDH-deficient tumors were wild-type for *KIT* and *PDGFRA*, *SDHB* negative and demonstrated IGF1R overexpression. The three neurofibromatosis-related tumors were SDHB positive and IGF1R negative. Of the 40 unselected GISTs, five were wild-type for *KIT* and *PDGFRA* in the selected exons. Two of the wild-type GISTs were SDHB negative and showed IGF1R overexpression; three were SDHB positive and IGF1R negative. There are still some shortcomings in their study; for example, the exon 12 and exon 14 of *PDGFRA* were not sequenced, and their sample size is very small. However, when Corless *et al*.^[53]

sequenced 1105 GISTs, only 11 (1%) were found to harbor *PDGFRA* exon 12 mutations, and only 3 (0.3%) were found to harbor exon 14 mutations^[53]. Therefore, the results are unlikely to affect their basic conclusion that IGF1R overexpression is a feature of SDH-deficient GISTs. The limited sample size was corrected by a study carried out by Lasota *et al*.^[54]; in their study, IGF1R expression was examined immunohistochemically in 1078 well-characterized GISTs representing different clinicogenetic categories and 103 non-GIST gastrointestinal tumors. IGF1R expression was detected in 71/80 (89%) of SDH-deficient GISTs (SDHB-negative GISTs), but only in 9/625 (1%) of the SDHB-positive gastric GISTs. In addition, several studies support this conclusion, showing that IGF1R is highly expressed in wild-type GISTs and pediatric GISTs^[55-57]. However, there are still some differences in these studies; the study by Pantaleo MA and colleagues^[56] showed that wild-type GISTs have a higher level of amplification of the *IGF1R* gene compared with mutants and that there are no mutations in the *IGF1R* gene in wild-type GISTs. However, another study by Janeway *et al*.^[57] indicated that *IGF1R* gene amplification was not detected in pediatric wild-type GISTs. Two immunohistochemical studies by Rios-Moreno *et al*.^[58] and Braconi *et al*.^[59] are discordant with the former findings. In Rios-Moreno's study, among the 22 IGF1R-positive samples examined, 82% (18/22) had a *KIT* mutation, 14% (3/22) had a *PDGFRA* mutation, and only 4% (1/22)

were wild-type *KIT/PDGFR*A. In Braconi's study, immunohistochemistry was performed on 13 wild-type GISTs and 81 mutant GISTs to detect the expression of IGF1R. They found that IGF1R was strongly expressed in the cytoplasm of all GISTs. However, both of the studies used a polyclonal anti-IGF1R antibody for immunohistochemistry. This polyclonal anti-IGF1R antibody has now been shown to lack specificity for this receptor. For example, it can produce multiple non-specific bands on Western blot and stain positively in cell lines derived from *IGF1R* knockout mice^[60]. Therefore, it is likely that the non-specific (false-positive) staining of the polyclonal anti-IGF1R antibody used in the previous two studies accounts for the apparent discrepancy. Recently, Nannini *et al.*^[61] found that IGF1R was upregulated in all patients harboring SDH mutations or displaying SDH dysfunction, with respect to *KIT/PDGFR*A wild-type GISTs without SDH mutations. This report confirmed that IGF1R overexpression in *KIT/PDGFR*A wild-type GISTs could be driven by the loss of function of the SDH mitochondrial complex.

Braconi *et al.*^[59] investigated the immunohistochemical expression of IGF1 and IGF2 in 94 samples of GISTs and found that strong IGF expression significantly correlated with a higher mitotic index, larger tumor size, a higher risk for metastases and relapsed GISTs. Another study by Gu *et al.*^[62] showed similar results. Although the samples of these two studies were unselected GISTs rather than SDH-deficient GISTs, they are able to clarify the important role of IGF in GIST development. Consequently, the IGF family (IGF and its receptors) provides valuable clues for the treatment of GISTs.

As early as 2004, Antonescu *et al.*^[63] determined the variation of gene expression in 28 GIST samples from 24 patients and showed that gene expression was different between wild-type and mutant GISTs. Strong gene expression of vascular endothelial growth factor (VEGF), macrophage colony stimulating factor, and BCL2 was found in the wild-type group while overexpression of these genes was not observed in mutant GISTs. Variations in gene expression between spindle and epithelioid GIST cells also follow a similar pattern. Most of the gastric epithelioid GISTs lack *KIT* mutations, whereas genes associated with the epithelial cell phenotype (TP73L and Keratin1) were detectable. Compared with the spindle cells, genes involved in apoptosis (BCL2 and Caspase 10), angiogenesis (VEGF) and proliferation (PDGF1) were up-regulated in the epithelioid GIST cells. In addition, there was a remarkable difference in gene expression between stomach and small bowel GISTs. A number of genes involved in muscle contraction and development were found to be differentially expressed in these two anatomical sites. Because SDH-deficient GISTs were not well recognized at that time, we can speculate that these differences are most likely caused by SDH dysfunction.

Additionally, positive *KIT*, DOG1/Ano1 and CD34 were observed in the majority of SDH-deficient GISTs by immunohistochemistry while muscle markers (SMA and desmin) were rarely detected. None of the tumors were S100 protein positive^[12].

POSSIBLE MECHANISM OF SDH DEFICIENCY

Although the etiology of SDH deficiency in these tumors remains unclear, the relationship between SDH deficiency and *SDH* gene mutation is well known. Many studies have identified the germline mutations of *SDHB* (IVS1+1 G→T or c.72+1G>T; IVS4+1G>C or c.423+1G>C; c.423+1 G→C; c.45_46insCC), *SDHC* (c.43+1 C→T; IVS5+1 G→A), and *SDHD* (c.57delG) in patients with Carney-Stratakis syndrome^[21,22]. It is worth noting that these patients did not have *KIT* or *PDGFR*A mutations. Recently, despite the complexity of its locus (15 exons) and the presence of three pseudogenes, *SDHA* was analyzed and the nonsense or missense mutations in exon 2 (c.91C>T), exon 5 (c.553C>T), exon 8 (c.1043-1055del; c.688delG; c.985C4T), exon 9 (c.1151C>G), exon 11 (c.1534C4T) and exon 13 (c.1765C>T) were found in *KIT/PDGFR*A WT GIST patients^[23,25].

Celestino *et al.*^[64] studied a series of 25 apparently sporadic primary *KIT/PDGFR*A/*BRAF* wild-type GISTs occurring in patients without personal or familial history of paragangliomas (PGLs), finding that *SDHB* expression was absent in 20% of wild-type GISTs while *SDHB* germline mutations were detected in 12% of wild-type GISTs. The type of *SDHB* germline mutations includes promoter region or exon 1 deletion and point mutation. However, there are still many SDH-deficient GISTs that have no mutations in the gene coding for SDH subunits. To explore additional pathogenetic mechanisms in these GISTs, Kelly *et al.*^[65] investigated the post-transcriptional regulation of these tumors by conducting microRNA (miRNA) profiling of a mixed cohort of 73 cases, which included 18 gastric pediatric wild-type GISTs, 25 (20 gastric, 4 small bowel and 1 retroperitoneal) adult wild-type GISTs and 30 gastric adult mutant GISTs. Using this approach, they identified a cluster of miRNAs on 14q32 that show significantly different expression patterns among GISTs, which appears to be explained at least in part by differential allelic methylation of this imprinted region. Interestingly, some wild-type GISTs lack SDH gene mutations but show either a marked reduction or an absence of *SDHB* protein expression by immunohistochemistry and a corresponding loss of respiratory chain complex II enzymatic activity. Furthermore, *SDHB*, *SDHC* and *SDHD* mRNA levels in these GISTs are comparable with those in *KIT*-mutant GISTs, which suggests that *SDHB* downregulation occurs at the level of protein translation^[4,62]. In addition, other possible mechanisms, including deficiency or

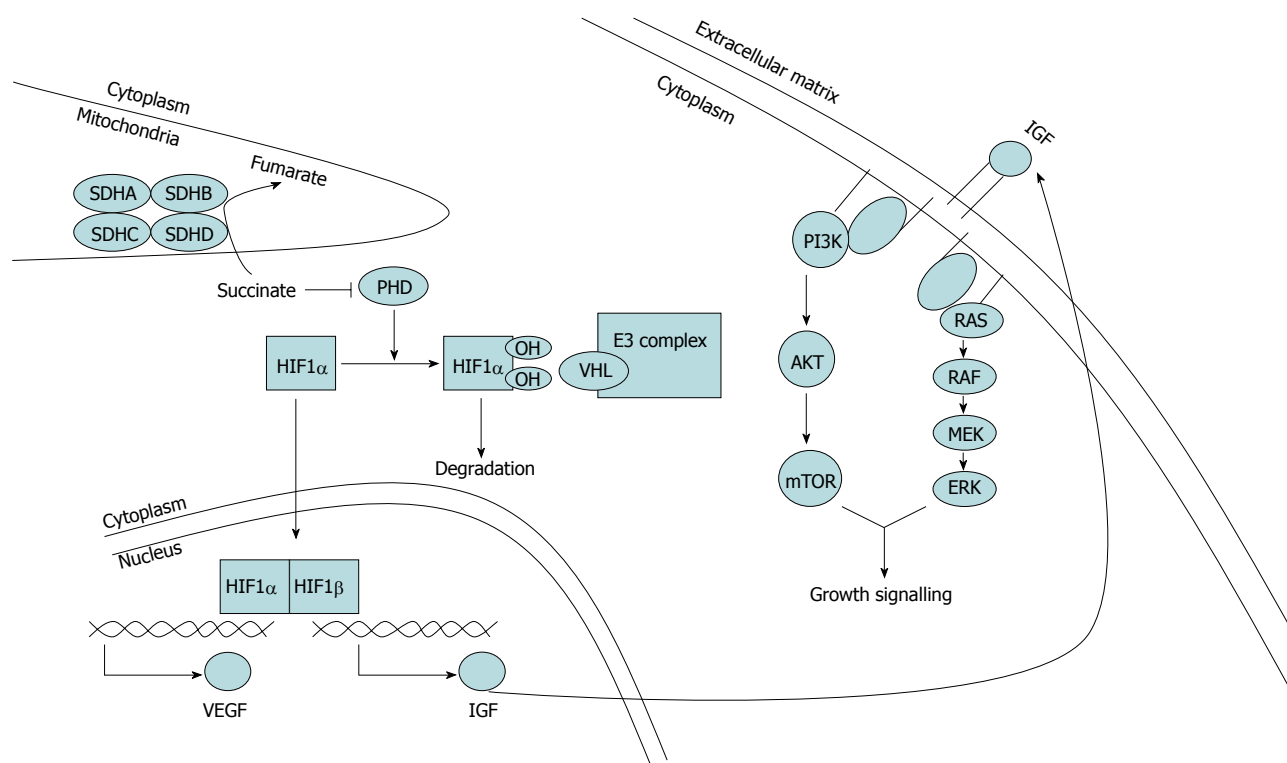


Figure 3 Role of succinate dehydrogenase deficiency in the tumorigenesis of succinate dehydrogenase-deficient gastrointestinal stromal tumors. SDH complex dysfunction within mitochondria leads to increased levels of succinate, which in turn inhibits PHD activity. Non-hydroxylated HIF1 α is resistant to VHL-mediated targeting for degradation and stimulates transcription of VEGF and IGF. SDH: Succinate dehydrogenase; HIF1 α : Hypoxia-inducible factor α ; PHD: Prolyl hydroxylase; E3 complex: Ubiquitin ligase complex; VHL: Von Hippel-Lindau; VEGF: Vascular endothelial growth factor; IGF: Insulin-like growth factor; PI3K: Phosphatidylinositol 3-kinase; mTOR: Mammalian target of rapamycin.

alterations in proteins involved in stabilization of the SDH complex, should be considered. For example, loss of function mutations in SDHAF2 can also result in the destabilization of the SDH complex and a loss of complex II activity^[66,67].

ROLE OF SDH DEFICIENCY IN THE TUMORIGENESIS OF SDH-DEFICIENT GISTS

Succinate dehydrogenase is localized in the inner mitochondrial membrane and consists of four subunit proteins (SDHA, SDHB, SDHC and SDHD)^[16]. The function of SDH is to act at the interphase of the tricarboxylic acid cycle and electron transport chain and catalyze the oxidation of succinate to fumarate^[18,19]. This complex is an important regulator of hypoxia-inducible factor 1 α (HIF1 α), a subunit of HIF1. HIF1, a heterodimer of HIF1 α and HIF1 β , is a transcriptional activator of IGF2 and VEGF^[68,69]. In normoxic conditions, HIF1 α undergoes proteasomal degradation after ubiquitination by a ubiquitin ligase complex targeted at HIF1 α by the VHL protein^[70]. Binding of VHL to HIF1 α requires hydroxylation of proline residues in HIF1 α , which is mediated by the oxygen-dependent activity of a prolyl hydroxylase-domain (PHD) protein^[71], an enzyme that also

converts a ketoglutarate to succinate^[68]. Because the activity of PHD is oxygen dependent, hypoxia leads to decreased activity of PHD, which, in turn, results in reducing the hydroxylation of HIF1 α , a lack of VHL-mediated ubiquitination of HIF1 α , and ultimately stabilization (and hence accumulation) of HIF1 α ^[72]. When HIF1 α accumulates, it migrates to the nucleus where it dimerizes with HIF1 β to form an active transcription factor that induces expression of genes involved in glycolysis and angiogenesis (including IGF and VEGF)^[73,74], thereby promoting the adaptation of cells to low oxygen by inducing neovascularization and glycolysis. The dysfunction of SDH leads to the accumulation of succinate, which in turn inhibits PHD activity, induces a pseudohypoxia phenomenon and promotes downstream gene expression (Figure 3). Consistent with the previous findings, HIF1 α and VEGF expression is higher in wild-type GISTs and SDH-deficient tumors than in KIT-mutant GISTs^[75-77]. Therefore, the upregulation of HIF1 α may lead to enhanced growth signaling by increasing the combination of IGF and VEGF with their receptors (IGF1R and VEGFR) in SDH-deficient GISTs^[2,68,78]. In addition, other factors may contribute to the development of SDH-deficient tumors. For example, SDH mutations may result in redox stress due to the increased production of reactive oxygen species, which is considered the oncogenic trigger of many

tumors^[76,79,80].

POSSIBLE MECHANISM OF TUMORIGENESIS IN SDH-DEFICIENT GISTS DRIVEN BY IGF1R

Previous studies have demonstrated that wild-type GISTs are characterized by IGF1R overexpression at both the mRNA and protein levels without genomic amplification^[55-57]. It has been reported that SDH dysfunction may be related to tumorigenesis in GISTs *via* induction of a pseudohypoxic pathway involving HIF1 α , which exerts its proliferative effects by activating key receptor tyrosine kinases such as IGF1R^[68,81-84]. Therefore, it would be reasonable to speculate that the overexpression of IGF1R in SDH-deficient tumors occurs as a consequence of HIF1 α activation induced by SDH dysfunction^[61].

IGF1R is a transmembrane receptor that is activated through autophosphorylation once ligands (IGF1 or IGF2) bind, thereby leading to the activation of the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades. In the PI3K pathway, AKT is phosphorylated by PI3K indirectly, which can promote cell survival *via* multiple effectors. AKT effectors that regulate growth include mammalian target of rapamycin (mTOR) within a complex, which enhances the translation of proteins involved in proliferation. Meanwhile, in a parallel pathway, the sequential activation of RAS, RAF, and MAPK isoforms ERKs, results in the transcription of genes that drive growth^[85,86] (Figure 3). The overexpression of IGF1R has been identified in several tumor types and because of its role in cancer cell metabolism and the potential relevance to the survival of malignant cells, IGF1R has become a target for anticancer therapy^[87-89]. In addition to IGF1R overexpression, IGF2 can exert its mitogenic and anti-apoptotic effects by binding to the IGF1R and the insulin receptor (IR) isoform A. In addition, there is evidence that IGF2 can lead to an autocrine stimulation loop in several tumor types^[90-93]. Moreover, Rikhof *et al.*^[94] proved that IGF2 is commonly expressed in GISTs, suggesting that secreted IGF2 is involved in the pathogenesis of GISTs by providing a pro-survival signal in an autocrine manner.

TREATMENT OF SDH-DEFICIENT GISTS

GISTs are resistant to traditional chemotherapy but are responsive to the tyrosine kinase inhibitor imatinib^[95,96]. However, GISTs that are deficient in succinate dehydrogenase generally respond poorly to imatinib because of a lack of activating tyrosine kinase mutations^[7,95]. Consequently, it is of great importance to develop new therapeutic targets and novel specific drugs.

Because IGF1R overexpression is a feature of SDH-deficient GISTs, it seems to be a promising target for anticancer therapy. Currently, it is increasingly acknowledged that IGF1R is able to serve as a potential target for the treatment of various tumors. There are three target strategies: anti-receptor antibodies, anti-ligand antibodies and small-molecule receptor kinase inhibitors. All of these strategies have been used to target the IGF1R family, and certain drug candidates from each of classes were considered promising enough in preclinical models to be taken forwards to clinical trials^[97,98].

All of the anti-receptor antibodies were designed to spare the insulin receptors because they all interfere with ligand binding to the IGF1R. Although they lack interference with insulin binding, the use of these antibodies is associated with dangerous side effects, such as hyperglycaemia and hyperinsulinaemia^[97]. Furthermore, because the pituitary attempts to compensate for the perceived lack of IGF biological activity, the use of these agents results in a remarkable increase in growth hormone (GH) secretion. This leads not only to increases in circulating IGF levels but also to insulin resistance induced by GH, which is responsible for the observed hyperglycaemia and hyperinsulinaemia in treated patients^[97]. In addition, IGF (both IGF1 and IGF2) has been shown to increase islet growth^[99]. When IGF1R is blocked, this effect is enhanced by the increased free IGF. It is also reported that IGF1 and GH could induce β -cell proliferation^[100]. Similarly, the blockade of IGF1R is likely to promote β -cell proliferation. Thus, these mechanisms may further contribute to the side effects when using anti-receptor antibodies.

Anti-ligand antibodies have a high affinity against both IGF1 and IGF2. Consequently, ligand-antibody complexes would replace ligand-binding protein complexes in circulation (IGFBPs normally bind greater than 90% of circulating IGFs), resulting in high levels of free IGFBPs. There is evidence that free IGFBPs have antiproliferative activity that is independent of their IGF-binding capacity^[101]. In addition, the autocrine loop of IGF2 cannot be interrupted by IGF1R-specific antibodies but could be inhibited by ligand-specific antibodies, leading to the reduction of the secretion of IGF. The small-molecule tyrosine kinase inhibitors tend to inhibit all members of the IGF1R family *in vivo*. However, such agents do not cause severe metabolic toxicity. At the dosages used, insulin receptor signaling is incompletely inactivated. Additionally, it seems that the drug concentrations in muscle are fairly low, which is a major insulin-stimulated glucose disposition site. Therefore, the function of the insulin receptor is fairly intact, leading to a modest, rather than a severe effect of these small-molecule kinase inhibitors on systemic glucose metabolism.

However, several studies suggest that these anti-neoplastic strategies did not work in all GISTs patients. Although several early phase clinical trials of the use

of IGF1R-specific antibodies for cancer treatment raised enthusiasm, the results of the initial phase III in unselected patients have proven disappointing^[102]. Therefore, comprehensive clinical trials and reasonable therapeutic targets for IGF1R remain to be carried out *in vivo* in the future.

Additionally, there is evidence that the PI3K-mTOR signaling pathway is one of the most important pathways in the growth of GIST cells^[103]. Multiple medications targeting this pathway, such as Perifosine and Everolimus, are in clinical development^[104]. However, as described in previous reviews, these targets are downstream not only of the IGF receptor but also of other receptor tyrosine kinases^[102]. Thus, targeting these signaling nodes has the potential to not only inactivate certain important parts of the signaling networks downstream of the IGF family but also to decrease pivotal, proliferative survival signals that are initiated by other receptor tyrosine kinases. However, no drug candidates are expected to be tumor-specific, and all of them tend to inhibit downstream signaling of IGF family members in normal tissues. PI3K or AKT inhibition would be expected to lead to initial hyperglycemia and, secondarily, to compensatory hyperinsulinaemia. Metformin, a biguanide that is commonly used in the treatment of type 2 diabetes, can lower blood glucose by inhibiting gluconeogenesis and, secondarily, can reduce hyperinsulinaemia. In addition to the treatment of hyperglycemia and hyperinsulinaemia that are associated with IGF1R-targeting agents or PI3K inhibitors, metformin could also exert antineoplastic effects toward some tumors. Several experimental studies have demonstrated that metformin can reduce insulin-stimulated tumor growth *in vivo*. On one hand, it can reduce insulin levels and insulin receptor activation; on the other hand, it can decrease the effect of a high-energy diet on phosphorylation of AKT and the expression of fatty acid synthase^[105,106]. Consequently, combining PI3K pathway inactivators or IGF family specific inhibitors with metformin seems to be a promising strategy for antineoplastic therapy, resulting in better treatment and fewer adverse effects.

Moreover, VEGFR targeted drugs, such as sunitinib, motesanib, sorafenib, regorafenib, vatalanib, pazopanib and the monoclonal antibody bevacizumab, have the potential to decrease tumor growth by inhibition of angiogenesis^[4]. Sunitinib maleate (sunitinib malate; SU11248; SUTENT) is a small-molecule inhibitor of multiple receptor tyrosine kinases involved in cancer, including VEGFR, PDGFR and the KIT receptor^[107]. It was approved by the US Food and Drug Administration for the treatment of GISTs after disease progression or intolerant to imatinib mesylate and advanced renal-cell carcinoma in January 2006. In addition, many studies have shown that for patients with imatinib-resistant/intolerant GIST, continuous daily sunitinib dosing appears to be an active alternative dosing strategy with acceptable safety^[108].

CONCLUSION

SDH-deficient GISTs have gained increasing attention in recent years, but minimal molecular insight into its tumorigenesis and target therapy exists. In this review, we identified the features of SDH-deficient GISTs and further illustrated the possible mechanisms of tumorigenesis and clinical treatment of such GISTs. In SDH-deficient GISTs, SDH inactivation leads to the accumulation of HIF-1 α , which dimerizes with HIF-1 β in cell nuclei to form an intact HIF. HIF, acting as an active transcription factor, induces the expression of downstream genes, including IGF and VEGF. When these ligands combined with their receptors, a number of signaling pathways were activated and, finally, resulted in growth promotion and apoptosis inhibition of the tumor cells. Because of the important roles of IGF and VEGF in SDH-deficient GISTs, these molecules and their receptors have become potential therapeutic targets. However, many unknowns remain about such GISTs, including the reason why patients with SDH-deficient GISTs show differences in age, sex, position, phenotype, metastasis and pathological pattern compared with those with mutant GISTs. Moreover, the expression of IGF in SDH-deficient GISTs needs to be further studied. In addition, effective drugs and targeted therapy with better efficiency and fewer side effects need to be introduced *via* clinical trials. Finally, there certainly remains much to look forward to learning about this fascinating, newly recognized family of tumors.

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