Biology and targeted therapy of gastrointestinal stromal tumors

<Review>

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ABSTRACT

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Activating mutations of *KIT* or *PDGF receptor alpha (PDGFRA)* have been identified in the vast majority of GISTs. Most GISTs are sporadic and some kindred of familiar GIST with a germline mutation in *KIT* or *PDGFRA* have been reported. The inhibition of KIT or PDGFRA activity by the kinase inhibitor imatinib frequently results in dramatic clinical responses in advanced cases of GIST. The clinical response to imatinib therapy is correlated with the type of mutation in *KIT* and *PDGFRA*. Resistance to imatinib after an initial response has been reported and secondary point mutations in *KIT* or *PDGFRA* that confer imatinib resistance are the most common mechanisms responsible for an acquired resistance to imatinib. The determination of *KIT* and *PDGFRA* mutations would be useful in predicting the effect of imatinib. GISTs are the first solid tumors to respond well to targeted small molecular therapy. This review outlines the biology and targeted therapy of GISTs.

Key words : KIT, PDGFR, NF1, Tyrosine kinase inhibitor, Imatinib

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. It is generally thought that GISTs originate in smooth muscles and they are referred to as leiomyomas and leiomyosarcomas. However, electron microscopic and immunohistochemical studies related to smooth muscle differentiation have revealed inconsistencies in the above assumption. Thus, the term stromal tumor was introduced [1]. At present, GISTs are thought to originate from interstitial cells of Cajal (ICCs) or from stem cells that differentiate towards ICCs. In 1998, Hirota and Isozaki et al. reported that KIT is expressed in GISTs and that most of these tumors harbor activating mutations of *KIT* [2]. This discovery led to the development of therapies targeting KIT in the treatment of GISTs. This review outlines the biology and advances in the targeted

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therapy of GIST.

KIT or *PDGFRA* mutations in GIST

Approximately 80–85% of GISTs have activating mutations of *KIT*[3]. The proto-oncogene *KIT* was first identified as the cellular homologue of the oncogene *v-KIT*, isolated from the Hardy-Zuckerman 4 feline sarcoma virus[4]. The KIT protein is a transmembrane type III receptor tyrosine kinase, the ligand of which is stem cell factor (SCF). KIT consists of an extracellular region, containing five immunoglobulin-like motifs, and a cytoplasmic region, containing a juxtamembrane domain and two kinase domains (Fig. 1). A kinase insert sequence divides the kinase domain into an ATP binding region and a phosphotransferase region. KIT plays a crucial role in the development of melanocytes, erythrocytes, germ cells, mast cells and ICCs. ICCs that regulate gastrointestinal motor function are located in and near the circular muscle layer of the gastrointestinal tract. GISTs are thought to arise from ICCs or stem cells that differentiate towards ICCs.

The mutant KIT proteins in GISTs are constitutively activated in the absence of the KIT ligand, SCF (Fig. 1)[2]. Signal transduction pathways such as the PI3K/Akt and mitogen-activated protein kinase pathways have been implicated in the mediation of KIT-induced mitogenesis and its differentiated function[5, 6]. *KIT* mutations in GISTs in exons 9, 11, 13, and 17 of the gene have been reported, with the majority in the juxtamembrane domain (exon 11) (Fig. 2)[2, 3, 7–14]. *KIT* mutations in the extramembrane region (exon 9) were found in 13% of GISTs, while mutations (exons 13 and 17) in the two kinase domains are rare[3].

Approximately 30% of GISTs lacking *KIT* mutations have activating mutations in *PDGFRA* (Fig. 2)[15–17]. PDGFRA is a member of the same family of type III receptor tyrosine kinase as KIT and a high amino acid homology exists between PDGFRA and KIT. *PDGFRA* mutations were found in the juxtamembrane domain (exon 12), the kinase I domain (exon 14) and the kinase II domain (exon 18). *KIT* and *PDGFRA* mutations have been suggested to be alternative and mutually exclusive oncogenic mechanisms in GISTs and it has also been suggested that mutations in *KIT* and *PDGFRA* modulate the differential activation and expres-

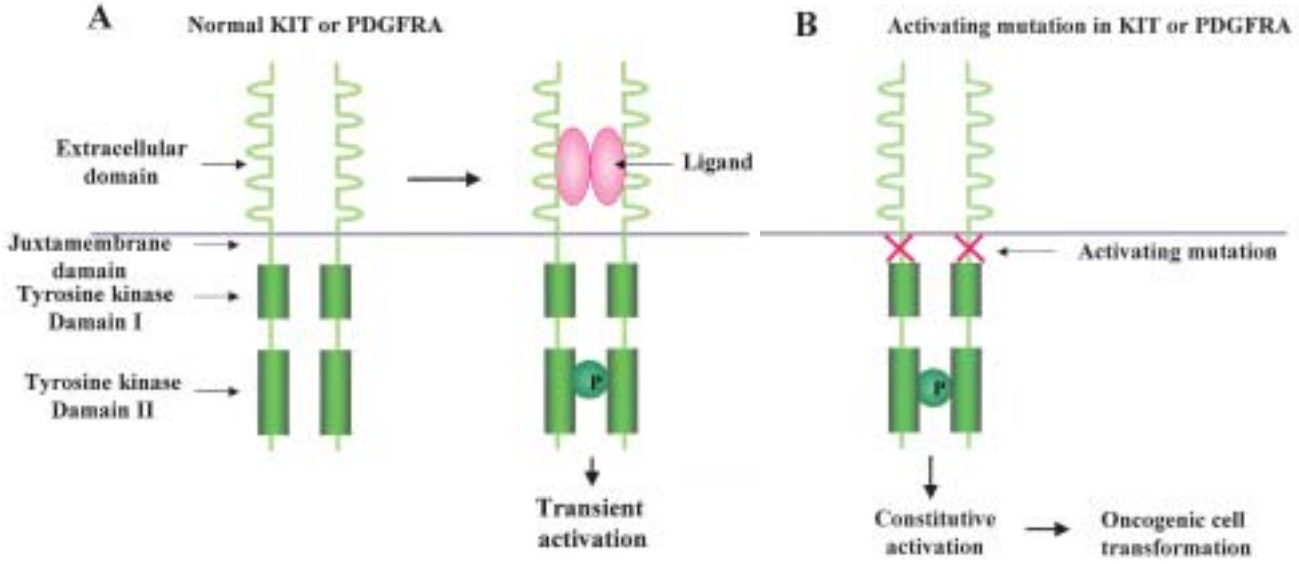

Fig. 1 Normal and abnormal KIT or PDGFRA signaling

A : Normal KIT or PDGFRA signaling

B : Constitutive activation of KIT or PDGFRA signaling in GISTs. Gain-of-function mutations result in ligand-independent KIT/PDGFRA activation of the kinase domain.

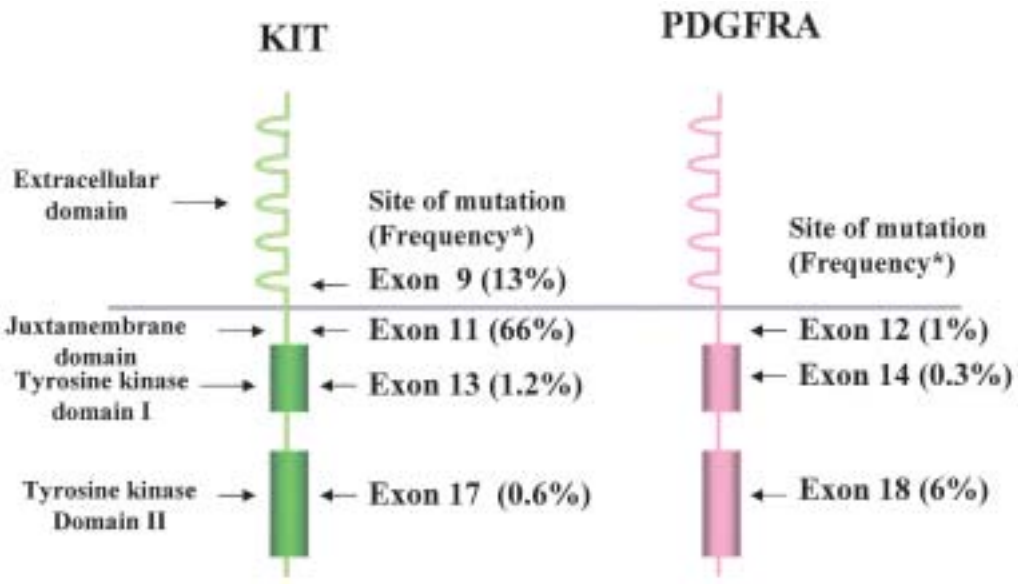

Fig. 2 Site of *KIT* and *PDGFRA* mutations in GISTs
＊: Based on data reported by Corrless *et al*.[3, 17]

sion of some types of genes[18].

Patients with NF1 (von Recklinghausen's neurofibromatosis type 1) are at increased risk of developing GISTs. Based on a Swedish study involving 70 NF1 patients, the incidence of GISTs in this population is approximately 7%[19]. Mutations in the *KIT* gene or the *PDGFRA* gene appear to be rare in GISTs from NF1 patients[20-23]. Interestingly, in NF1 patients, GISTs develop in the small intestine more frequently than the stomach, while GISTs of non-NF1 patients are most commonly found in the stomach. It is possible that the underlying mechanism of GIST carcinogenesis in NF1 patients is different from that in non-NF1 patients.

Familiar GISTs

Familial GISTs are rare autosomal dominant genetic disorders. Twelve kindred of familial GISTs with germline mutations in the *KIT* and *PDGFRA* genes have been reported [24-35] (Table 1), including 11 with *KIT* mutations and one with a *PDGFRA* mutation. Among 11 kindred with germline mutations of *KIT*, 7 had a *KIT* mutation in the juxtamembrane domain and the other kindred had *KIT* mutations in the extracellular domain, kinase domain I or II. Most mutations in kindred of the GIST family are identical to those found in sporadic GISTs. Skin hyperpigmentation, dyphagia, and mastocytosis were observed in some kindred of familial GISTs with germline mutations in the *KIT* gene. Diffuse hyperplasia of ICCs was detected in the myenteric plexus region of the gastrointestinal tract in some kindred with germline mutations in *KIT* [24-26, 33]. The diffuse ICC proliferation was found to be polyclonal, although the GISTs were monoclonal[36]. Recent data based on a knock-in mouse model suggests that germline *KIT* activation results in hyperplasia of ICCs[37]. A germline mutation of *KIT* may cause non-neoplastic hyperplasia of ICCs and a transition from hyperplasia to neoplasia. A case of familial GIST with dysphagia showed abnormal esophageal peristaltic movement, suggesting that hyperplasia of ICCs may be associated with esophageal motor disturbances[33]. Urticaria pigmentosa is the most common form of cutaneous mastocytosis. Urticaria pigmentosa or mastocytosis have been observed in 3 kindred of familial GISTs with a germline mutation in *KIT* [24, 29, 30]. In sporadic cases of systemic mastocytosis, a D 816V mutation in *KIT* is common[38], which has not been reported in GISTs. A germline mutation in *KIT* (A553D) was reported in a kindred of familial diffuse cutaneous mastocytosis [39].

Table 1 Associated symptoms in familial gastrointestinal stromal tumors with a germline mutation in *KIT* and *PDGFRA*

Family no.	Author (ref. no.)	Mutated gene	Site of mutation	Associated symptoms
1	Hartmann K (24)	*KIT*	Del419 (EC)	Mastocytosis, Dysphagia
2	Hirota S (25)	*KIT*	W557R (JM)	–
3	Robson ME(26)	*KIT*	W557R (JM)	Hyperpigmentation, Dysphagia
4	Nishida T (27)	*KIT*	Del559–560 (JM)	Hyperpigmentation
5	Maeyama H (28)	*KIT*	V559A (JM)	Hyperpigmentation
6	Beghini A (29)	*KIT*	V559A (JM)	Hyperpigmentation, Urticaria pigmentosa
7	Li FP (30)	*KIT*	V559A (JM)	Hyperpigmentation, Urticaria pigmentosa
8	Carballo M (31)	*KIT*	InsQL576–577 (JM)	–
9	Isozaki K (32)	*KIT*	K642E (TKI)	–
10	Hirota S (33)	*KIT*	D820Y (TKII)	Dysphagia
11	O'Riain C(34)	*KIT*	D820Y (TKII)	Dysphagia
12	Chompret A (35)	*PDGFRA*	D846Y (TKII)	–

EC: extracellular domain, JM: juxtamembrane domain, TKI: tyrosine kinase I domain, TKII: tyrosine kinase II domain

Prognostic factors of GIST

It is often difficult to predict the level of malignancy of GISTs. The presence of distant metastasis and/or direct invasion of adjacent organs, a large tumor size, and a high mitotic rate have been identified as unfavorable predictors for survival. The NIH consensus conference proposed a risk classification based on tumor size and histopathological mitotic count (Table 2)[40]. If the tumor is less than 5 cm in size and the mitotic count is below a 5/50 high power field, the risk of metastasis can be considered to be low.

Several studies have indicated that molecular alterations may serve as predictors of the clinical outcome in GISTs. Loss of the p16 protein[41], the *KIT* mutation type[42–45], telomerase activity[46], hypermethylation of the E-cadherin promoter[47], and the expression of a set of six genes: *CCNB1, CENP-F, FAK, HMG2, TSG101*, and ezrin[48] have been reported to be molecular markers that are indicative of a poorer prognosis for GISTs. The location of the *KIT* and *PDGFRA* mutations in GISTs has been reported to be associated with both the site of origin and the prognosis[45]. A significant association between *KIT* exon 9 mutations and an intesti-

Table 2 Proposed guidelines for defining the risk of aggressive behavior in gastrointestinal stromal tumors

Risk	Size (cm)	Mitotic index (per 50 HPF)
Very low	<2	<5
Low	2–5	<5
Intermediate	<5	6–10
	5–10	<5
High	>5	>5
	>10	Any mitotic rate
	Any size	>10

HPF: High-power field.
From Fletcher *et al* [37].

nal origin of GISTs, and between *PDGFRA* mutations and gastric GISTs has been reported. The 6 bp insertion mutation in *KIT* exon 9, resulting in the tandem duplication of amino acids Ala 502 and Tyr 503, was shown to define GISTs of intestinal origin with a more aggressive potential [43]. Internal tandem duplication in the 3' end of *KIT* exon 11 was reported to be associated with gastric GISTs with a more favorable outcome[44], while deletions that affect codons 557–558 of *KIT* exon 11 indicated a poor prognosis[45].

Molecularly targeted therapy of GIST with imatinib

Imatinib (imatinib mesylate, commercially available as Gleevec or Glivec, Novartis, Basel, Switzerland), formerly known as STI571, is a tyrosine kinase inhibitor developed for the treatment of chronic myeloid leukemia by targeting the BCR–ABL fusion protein responsible for leukemic transformations. Imatinib inhibits the kinase activities of KIT and the PDGF receptor[49, 50], by blocking the binding of ATP to these tyrosine kinases. Imatinib blocks the in vitro kinase activity of both wild-type KIT and a mutant KIT isoform commonly found in GISTs [51]. Conventional chemotherapy and radiation therapy is ineffective in the treatment of GIST. A patient with GIST metastatic to the liver was successfully treated with imatinib[52]. The success in treating the first GIST patient with imatinib quickly led to initial phase I/II studies, followed by phase III randomized trials with imatinib in patients with metastatic or unresectable GISTs[53–56]. The US–Finland multicenter trial (CSTI571B 2222) showed that the partial response rate (PR) and the stable disease rate (SD) were 54% and 28%, respectively, in patients with advanced GIST [54]. Other clinical studies also showed a high overall response rate and the phase III studies suggested an increase in progression-free and overall survival rates[55, 56]. Although the use of imatinib frequently results in long-term tumor shrinkage in metastatic GISTs, complete remission after imatinib treatment is rare. The 1-year results of a French phase III study of continuous versus intermittent imatinib treatment showed a rapid and frequent progression at 3 months in patients on the intermittent regimen[57]. The continuous imatinib regimen is therefore the recommended standard approach. Trials of adjuvant and neoadjuvant treatment of GISTs with imatinib are currently underway.

Treatment with imatinib is generally safe and well tolerated, although most patients experiance some mild to moderate adverse events[54–56]. The most common adverse events include anemia, edema, nausea, diarrhea, myalgia, fatigue, and skin rash[54–56]. Overall, the adverse effects of imatinib are similar to those reported for a large population of patients with chronic myeloid leukemia. Less than 2% of the patients were taken off treatment due side effects. Toxicity-related deaths occurred in 0.5% to 2% of the patients, mainly due to hemorrhage or hepatotoxicity. Gastrointestinal or intra-abdominal hemorrhages are generally thought to be related to tumor regeneration induced by imatinib.

Clinical response to imatinib based on the type of *KIT* and *PDGFRA* mutation

Correlative studies associated with one of the multicenter trials (CBTI571B 2222) showed that the clinical response to imatinib was correlated with the type of mutation of *KIT* and *PDGFRA* [8]. Patients with GIST harboring exon 11 *KIT* mutations had a significantly better response to imatinib (83.5%) than those with exon 9 *KIT* mutations (48.7%), and those without *KIT* or *PDGFRA* mutations (0%). Patients with a D842V mutation in *PDGFRA*, the most common activating *PDGFRA* mutation in GISTs, failed to respond to imatinib therapy. The *PDGFRA*-D842V mutation is confirmed to be an activation mutation with an attenuated sensitivity to imatinib[58]. These results indicate that the determination of *PDGFRA* mutations, in addition to *KIT* mutations, would be useful for predicting the effect of imatinib.

Mechanisms of imatinib resistance

Although most patients with GIST achieve a response to imatinib, many patients with GISTs develop imatinib resistance during a long-term treatment. The median time to progression has been reported to be about 24 months[56]. In patients with GIST who developed imatinib resistance, secondary mutations are often detectable in *KIT* or *PDGFRA* that are resistant to imatinib. These include V654A, D670I, D716N, D816G, D820E, D820Y, and N822K mutations in *KIT* and D842V mutation in *PDGFRA* (Fig. 3)[59-62]. D820Y and N822K mutations in *KIT* and a D 842V mutation in *PDGFRA* have been reported in GISTs that had not been treated with imatinib[8, 15, 16, 33], while the other mutations have not been reported previously in primary GISTs. Most of the secondary mutations are in *KIT* exons 17 and 13. This suggests that the acquisition of a secondary point mutation in *KIT* or *PDGFRA* results in the substitution of some residues at critical binding sites for imatinib. A secondary mutation in *BCR-ABL* is the most common mechanism of imatinib resistance in the treatment of chronic myeloid leukemia.

Imatinib resistance is a clinically crucial problem in the treatment of GIST. New molecularly targeted therapies are currently under development for GIST patients who are refractory to imatinib. SU11248 is a multi-targeted tyrosine kinase inhibitor of KIT, FLT3, PDGFR, and vascular endothelial growth factor receptor [63]. The use of SU11248 results in clinical benefits in a majority of patients with GIST who are refractory to imatinib[64]. A phase III randomized trial comparing SU11248 versus a placebo in these patients is currently underway. Other drugs that are being evaluated in imatinib-refractory patients include rapamycin analogue inhibitors, antisense oligonucleotides to bcl-2 mRNA, protein kinase C inhibitors, neutralizing antibodies against vascular endothelial growth factor, and several multikinase inhibitors [53].

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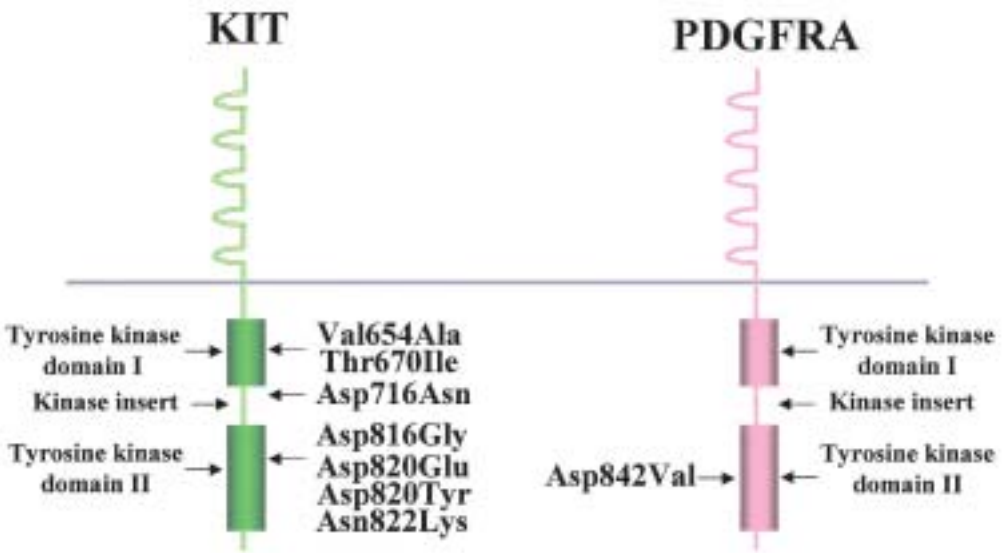

Fig. 3 Secondary mutations in *KIT* and *PDGFRA* that confer imatinib resistance[59-62]

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