Imatinib resistance and microcytic erythrocytosis in a Kit V558Δ;T669I/+ gatekeeper-mutant mouse model of gastrointestinal stromal tumor

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Table P1. Overview of phenotypes in mice with Kit mutations and their sensitivity to targeted kinase inhibition

<table>
<thead>
<tr>
<th>Kit kinase mutation status</th>
<th>Wild type</th>
<th>Loss of function</th>
<th>Primary gain of function (V558Δ allele)</th>
<th>Second-site double mutation (V558Δ;T669I allele)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial cells of Cajal</td>
<td>+</td>
<td>-</td>
<td>++ (hyperplasia)</td>
<td>+++ (hyperplasia)</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Mast cell numbers</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Red blood cell development</td>
<td>+</td>
<td>(macrocytic anemia)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Kinase sensitivity to imatinib/ dasatinib in vivo</td>
<td>+</td>
<td>NA</td>
<td>++</td>
<td>(microcytic erythrocytosis)</td>
</tr>
<tr>
<td>Kinase sensitivity to sunitinib/ sorafenib in vivo</td>
<td>+</td>
<td>NA</td>
<td>++</td>
<td>resistance</td>
</tr>
</tbody>
</table>

+, present/normal; ++, increased; ++++, greatly increased; - absent/impaired; NA, not applicable.

Targeted drug therapy enables the specific inhibition of enzymes involved in the pathogenesis of cancer. Protein kinases are critical regulators of cellular processes, including cell proliferation and cell survival. Oncogenic driver mutations are highly prevalent in protein kinases; therefore, protein kinases are logical targets for therapeutic intervention. Although the design of kinase inhibitors that display high degrees of specificity has met with many challenges, the prospect of their utility in reducing side effects commonly associated with standard chemotherapy continues to be a driving force behind their development. The first breakthrough drug to come out of this effort was imatinib, an inhibitor of the KIT and PDGF receptor tyrosine kinases and the BCR-ABL fusion protein, proteins involved in the development of several cancers, including gastrointestinal stromal tumor (GIST) and chronic myelogenous leukemia. Gain-of-function mutations in the KIT receptor are a hallmark of GIST. Today imatinib treatment is standard therapy for patients with GIST. Unfortunately, long-term treatment of GIST with imatinib is associated with the development of drug resistance, often as the result of the acquisition of a secondary mutation in the kinase domain of the KIT receptor. Given the clinical importance of imatinib resistance, the development of new strategies for the treatment of GIST is very important. In the current study, we have developed a mouse model for imatinib-resistant GIST.

The KIT receptor tyrosine kinase and its cognate ligand, Kit ligand, have critical functions in several cell lineages during embryonic development and in adult mammals. In hematopoiesis, KIT function is critical in the stem cell hierarchy and the development of red blood cells (erythropoiesis), mast cells, and platelets. A hallmark of mice carrying Kit loss-of-function mutations is a profound decrease in erythropoiesis (macrocytic anemia) and mast cell development. In addition, Kit is involved in several stages of melanogenesis and in primordial germ cells, spermatogenesis, and oogenesis. A major KIT-expressing cell in the gastrointestinal tract is the interstitial cell of Cajal (ICC). ICCs function as pacemakers of gut motility. In an elegant and pivotal study in 1992, Nishikawa and colleagues (1) showed that an antagonistic KIT antibody could interfere with autonomous gut movement in mice, essentially giving rise to a disorder resulting in the paralysis of the bowel, a condition known as "lethal paralytic ileus," and it was shown that mice with Kit loss-of-function mutations have impaired pacemaker activity and lack ICC networks.

Kit originally was discovered as an oncogene of a feline sarcoma virus (2). Later, gain-of-function mutations in the KIT receptor were reported in human mastocytosis, seminoma, and a subset of acute myelogenous leukemia, and in 1998, Kit mutations were also identified in GIST (3). GIST is the most common mesenchymal tumor of the gastrointestinal tract. GISTs express KIT, and they presumably derive from KIT-expressing ICC progenitors or from ICCs. Today, we know that the princi


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Principal genetic events responsible for the pathogenesis of GIST are gain-of-function mutations in the Kit gene, found predominantly in the juxtamembrane domain of the KIT receptor, which disrupt the conformational integrity of the protein and thus diminish the autoinhibitory function of the juxtamembrane domain. Mutations in the extracellular and kinase domains of KIT also have been described. Imatinib therapy is effective in GISTs with Kit-activating mutations in the juxtamembrane domain, some kinase-domain mutations, and extracellular domain mutations but is ineffective in tumors with Kit mutations that stabilize the active conformation of the kinase.

We previously produced a mouse model for GIST by introducing a Kit-activating mutation (KitV558Δ) originally identified in a case of familial GIST into the mouse Kit gene (4). Here we describe the generation of a mouse model for imatinib-resistant GIST that includes both the KitV558Δ mutation and a secondary KitT669I “gatekeeper” mutation, as a tool for developing therapeutic strategies for imatinib-resistant GIST and for investigating the consequences of KIT oncogenic signaling in other KIT-dependent cell systems, particularly pigment development (melanogenesis) and blood cell development (hematopoiesis). We found that KitV558Δ;T669I/+ mice exhibited increased ICC hyperplasia and more pronounced (mast cells) as well as distinct hematopoietic phenotypes (microcytic erythrocytosis) as compared with mice carrying only the primary Kit mutation (KitV558Δ/+)(Table P1). Importantly, although GIST lesions of KitV558Δ/+ and KitV558Δ;T669I/+ mice were similar in histology and oncogenic signaling, the KitV558Δ;T669I/+ mice were resistant to imatinib and to dasatinib tyrosine kinase-inhibitor therapy. This resistance could be overcome by treatment with the tyrosine kinase inhibitors sunitinib and sorafenib, supporting a rationale for using sunitinib as second-line therapy for imatinib-refractory GIST (Table P1). Interestingly, in the erythroid lineage, a polycythemia vera-like phenotype, with highly increased microcytic red blood cell numbers, was observed in KitV558Δ;T669I/+ mice. In agreement with our findings, the activity of the KitV558Δ;T669I kinase in vitro is nearly doubled (5), possibly explaining the Kit hyperactivity observed in the ICC, mast cell, and erythrocyte lineages in KitV558Δ;T669I/+ mice. Our results highlight the importance of a combination of factors, including the type of activation-mutation and cellular context, involved in determining mutant/oncogenic phenotypes in vivo. In summary, the KitV558Δ;T669I/+ mice provide an excellent tool for developing therapeutic strategies for imatinib-resistant GIST and for investigating the consequences of KIT oncogenic signaling in other KIT-dependent cell systems, particularly pigment development (melanogenesis) and blood cell development (hematopoiesis).