Targeting Disease Persistence in Gastrointestinal Stromal Tumors

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SUMMARY

Gastrointestinal stromal tumors (GISTs) represent 20%–40% of human sarcomas. Although approximately half of GISTs are cured by surgery, prognosis of advanced disease used to be poor due to the high resistance of these tumors to conventional chemo- and radiotherapy. The introduction of molecularly targeted therapy (e.g., with imatinib mesylate) following the discovery of the role of oncogenic mutations in the receptor tyrosine kinases KIT and platelet-derived growth factor α (PDGFRA) significantly increased patient survival. However, GIST cells persist in 95%–97% of imatinib-treated patients who eventually progress and die of the disease because of the emergence of clones with drug-resistant mutations. Because these secondary mutations are highly heterogeneous, even second- and third-line drugs that are effective against certain genotypes have only moderately increased progression-free survival. Consequently, alternative strategies such as targeting molecular mechanisms underlying disease persistence should be considered. We reviewed recently discovered cell-autonomous and microenvironmental mechanisms that could promote the survival of GIST cells in the presence of tyrosine kinase inhibitor therapy. We particularly focused on the potential role of adult precursors for interstitial cells of Cajal (ICCs), the normal counterpart of GISTs. ICC precursors share phenotypic characteristics with cells that emerge in a subset of patients treated with imatinib and in young patients with GIST characterized by loss of succinate dehydrogenase complex proteins and lack of KIT or PDGFRA mutations. Eradication of residual GIST cells and cure of GIST will likely require individualized combinations of several approaches tailored to tumor genotype and phenotype.

Significance

Gastrointestinal stromal tumors (GISTs) are one of the most common connective tissue cancers. Most GISTs that cannot be cured by surgery respond to molecularly targeted therapy (e.g., with imatinib); however, tumor cells persist in almost all patients and eventually acquire drug-resistant mutations. Several mechanisms contribute to the survival of GIST cells in the presence of imatinib, including the activation of “escape” mechanisms and the selection of stem-like cells that are not dependent on the expression of the drug targets for survival. Eradication of residual GIST cells and cure of GIST will likely require individualized combinations of several approaches tailored to the genetic makeup and other characteristics of the tumors.

GASTROINTESTINAL STROMAL TUMORS AND INTERSTITIAL CELLS OF CAJAL

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract [1,2]. With an estimated annual incidence of 3,300–6,000, GISTs represent a substantial proportion of the ∼15,000 bone and soft tissue sarcomas diagnosed in the U.S. each year [3]. Based on morphological, ultrastructural, and immunophenotypic characteristics including the expression of the receptor tyrosine kinase (RTK) KIT in ∼95% of cases [4], GISTs are thought to originate from stem cells that differentiate toward the lineage of interstitial cells of Cajal (ICCs) [5–8]. ICCs are KIT-expressing (KIT+), mesodermally derived mesenchymal cells that occur throughout the gut tunica muscularis [9]. In concert with the enteric nervous system and smooth muscle cells, ICCs govern gastrointestinal motor functions by generating electrical pacemaker activity, mediating cholinergic excitatory and nitric inhibitory neuromuscular neurotransmission, and by setting smooth muscle membrane potential and tone [9–12]. ICCs and most GISTs, including ∼50% of KIT-negative (KIT−) GISTs, also share expression of the calcium-activated chloride channel anoctamin 1 (ANO1; also known as TMEM16A and discovered on GIST-1 [DOG1]) [8, 13–17]. However, the relationship of KIT−ANO1− GIST to the ICC lineage cannot be ascertained based on immunohistochemical criteria. A subset of these tumors have been proposed to arise, possibly due to activated hedgehog signaling [18], from interstitial cells expressing platelet-derived growth factor α (PDGFRA); these cells mediate purinergic inhibitory neuromuscular neurotransmission [9]. The majority of GISTs arise on the basis of mutually exclusive, most often heterozygous activating mutations in KIT (75%–80%) [6] or PDGFRA (<10%) [19], which encodes a closely related RTK often coexpressed with KIT [20, 21]. In rare cases, GIST develops...
in patients carrying germline autosomal dominant KIT or PDGFRA mutations (familial GIST syndrome) [22]. Constitutively activated KIT cooperates with ET1V, a master regulator of the ICC transcription program, to bring about GIST oncogenesis [23]. RTK-mutant GIST may be composed of spindle-shaped and/or epithelioid cells, have no predilection for either sex, mainly occur in patients older than 50 years, and can arise anywhere in the gastrointestinal tract and within the mesentery, omentum, retroperitoneum, and pelvis [4]. Molecular alterations underlying clinical progression of GIST include chromosomal losses or gains and deletions, mutations, or downregulation of cell cycle regulator genes [22, 24]. Intragenic deletion of dystrophin, specifically affecting larger isoforms, often underlies aggressive, metastatic behavior [25]. GISTs that lack KIT or PDGFRA mutations (usually, and most often erroneously, termed “wild-type” GISTs; 10%–15%) may contain driver mutations in BRAF, HRAS, or NRAS or may develop in patients with neurofibromatosis type 1 [26] carrying germline autosomal dominant mutations in NF1 [22, 27]. These tumors are morphologically and clinically indistinguishable from RTK-mutant GISTs including expression and activation of KIT. A small subset of tumors lacking KIT or PDGFRA mutations and the majority (70%–75%) of pediatric GISTs, which represent only 1%–2% of all cases, display unique clinicopathological features including a strong predilection toward female sex, predominant gastric origin, presentation as multifocal nodular growths, epithelioid morphology, and low frequency of cytogenetic aberrations. Importantly, this class of GIST shows increased expression of insulin-like growth factor 1 receptor (IGF1R) [27–31] accompanied by activation of downstream signaling pathways that IGF1R shares with KIT [31]. A key unifying characteristic of these tumors is the loss of mitochondrial succinate dehydrogenase complex iron sulfur subunit B protein (SDHB) [22, 27, 32]. SDHB loss, in turn, causes reduced SDH respiratory complex II enzymatic activity (pseudohypoxia), succinate accumulation, stabilization of hypoxia-inducible factor 1α, and transcriptional activation of hypoxia-inducible genes including vascular endothelial growth factors and IGF2. Furthermore, the reduced α-ketoglutarate:succinate ratios have also been shown to cause DNA hypermethylation, with concomitant reduction of DNA hydroxymethylation likely reflecting substrate-level inhibition of ten-eleven translocation (TET) family methylcytosine dioxygenases [33]. In GIST, SDHB depletion can arise from deleterious mutations leading to biallelic inactivation of SDHA in sporadic cases [27, 34], autosomal dominant SDHB-D mutations in familial cases with associated paragangliomas (Carney-Stratakis syndrome) [35, 36], or epigenetic repression of SDHC transcription from DNA hypermethylation in Carney triad [37, 38]. The latter is a nonfamilial syndrome involving synchronous or metachronous occurrence of GISTs, paragangliomas, and pulmonary chondromas [39]. Even when they occur in adults, SDHB-negative GISTs are characterized by early onset with median age of ~18–22 years [27]. Although pediatric GISTs often metastasize, they tend to grow more slowly [22].

**DISEASE PERSISTENCE AND THERAPY RESISTANCE IN GIST**

The standard of care and the only potentially curative therapy for patients with a primary localized GIST is surgery [40]; however, approximately 40% of patients develop tumor recurrence within 5 years and eventually die of the disease [41]. Because GIST is highly resistant to conventional chemo- or radiotherapy, RTK inhibitors are the mainstay of treatment for advanced GIST. Front-line treatment with imatinib, a competitive inhibitor of ATP binding to KIT, PDGFRA, ABL, and BCR-ABL kinases, can achieve disease control in 70%–85% of patients with KIT+ advanced GIST and median progression-free survival of >5 years [22]. Approximately 10% of patients have primary resistance to RTK inhibitors, and that mainly reflects the type of the driving mutation. Resistance developing after an initial benefit is mainly the result of acquired, drug-resistant mutations occurring almost exclusively in the same gene and allele as the primary oncogenic mutation [42]. Unfortunately, resistance mutations show considerable heterogeneity, even within different areas of the same tumor; therefore, even second- and third-line drugs (e.g., sunitinib, regorafenib) that can potently inhibit certain secondary mutations have increased median progression-free survival by only 5–6 months [43].

In patients who respond to imatinib, substantial reduction in tumor size occurs as a result of apoptosis, consistent with the unique dependence of KIT-mutant GIST on oncogenic KIT signaling, termed “oncogene addiction” [44]. However, although long-term disease control lasting more than a decade can be achieved in some patients, RTK inhibitors fail to eradicate GIST cells in 95%–97% of patients who eventually progress and succumb to the disease [22]. The surviving cells are metabolically quiescent and do not proliferate [45, 46]; however, their exit from the cell cycle is reversible, as indicated by in vitro data [47] and the increased rate of progression in patients in whom treatment was discontinued [22], necessitating lifelong therapy. The significance of GIST persistence despite continuing RTK inhibitor therapy is that it sets the stage for the selection of clones with secondary drug-resistant mutations, which cannot be effectively controlled by current pharmacological approaches [43]. Therefore, it can be argued that targeting disease persistence should take precedence over the development of additional pharmacological agents against secondary mutations.

**CELL-AUTONOMOUS MECHANISMS OF GIST PERSISTENCE**

The persistence during RTK inhibitor therapy of GIST cells carrying imatinib-sensitive mutations could result from “escape” mechanisms expressed by the tumor cells. Alternatively, a pre-existing subset of cells not addicted to oncogenic RTK signaling, for example, due to lack of significant expression of the mutant receptor, could selectively survive the treatment. In six studies that investigated KIT expression in patients who underwent imatinib or sunitinib treatment prior to surgery, 15 of 131 samples lacked KIT expression detectable by conventional immunohistochemistry [46, 48–52] (further samples studied in [46] displayed reduced KIT expression [KITlow/−]), whereas the remainder showed no obvious phenotypic change. Thus, both mechanisms may contribute to GIST persistence. Typically, the cells with low or no expression of KIT (KITlow/−) had epithelioid morphology [46, 50, 52], which also predominates in untreated KIT+ GISTs [53]. Previously, we described a rare Kitlow/− cell type with nondescript epithelioid morphology in the tunica muscularis of postnatal mice and demonstrated their ability to self-renew and differentiate into ICCs in cell culture, in tissue explants, and following allogeneic transplantation in vivo.
Figure 1. Hypothetical stem cell model of GIST persistence and acquired therapy resistance. Light blue circles: KIT-independent precursor cells carrying imatinib-sensitive KIT mutation but expressing very little or no KIT protein (KIT\textsubscript{low/−}). Dark blue circles: KIT\textsuperscript{+} cells arising from the KIT\textsubscript{low/−} cells carrying imatinib-sensitive KIT mutation. Open circles: dead cells. Pink circles: KIT\textsubscript{low/−}, KIT-independent precursors with acquired secondary imatinib-resistant mutation. Red circles: KIT\textsuperscript{−} cells differentiated from the KIT\textsubscript{low/−} precursors with secondary imatinib-resistant mutation. Filled arrow: imatinib treatment. Open arrow: cessation of imatinib treatment. Abbreviations: GIST, gastrointestinal stromal tumor; KIT\textsuperscript{+}, cells with low or no expression of KIT; KIT\textsuperscript{−}, cells expressing KIT.

[7, 8, 54]. In mice carrying germline oncogenic KIT mutation, the population of these ICC stem cells (ICC-SCs) was dramatically increased, paralleling ICC hyperplasia [8]. Furthermore, clonally derived, spontaneously transformed ICC-SCs gave rise to GIST-like tumors in immunocompromised mice [8]. Besides epithelioid, Kit\textsuperscript{low} cells, these tumors contained spindle-shaped or ICC-like Kit\textsuperscript{−} cells, indicating that both phenotypes can arise from the same Kit\textsuperscript{low/−} precursor. Importantly, despite the competitive growth advantage that the Kit mutation seemed to confer on these Kit\textsuperscript{low/−} cells over their non-neoplastic counterparts, neither normal nor transformed (including Kit-mutant) ICC-SCs were dependent on Kit signaling for survival, as indicated by their low sensitivity to imatinib. These findings are consistent with a GIST model in which a small number of mutated ICC-SCs gives rise to Kit\textsuperscript{+} cells, representing the bulk of the tumors (Fig. 1). Although RTK inhibition can control the differentiation, proliferation, and survival of Kit\textsuperscript{−} GIST cells, it may not eradicate the inherently imatinib-resistant Kit\textsuperscript{low/−} stem cell pool from which the tumor is re-established following the cessation of therapy. Acquisition of an imatinib-resistant mutation by the surviving precursors would again permit their differentiation into Kit-expressing cells and uncontrolled GIST growth [8]. It follows that stimulation of Kit expression in the surviving Kit\textsuperscript{low/−} GIST precursors before the emergence of drug-resistant mutations could potentially restore the sensitivity of these cells to imatinib. Although this model bears remarkable similarities to the model proposed to underlie disease persistence in chronic myeloid leukemia [55, 56], its applicability to human GIST remains to be established.

In GIST cells dependent on imatinib-sensitive mutations, disease persistence may reflect incomplete apoptosis response to RTK inhibition [24]; however, relative to other solid tumors, loss-of-function mutations in the multifunctional proapoptotic protein p53 (TP53) are rare in GIST [24]. Instead, GIST cells may escape apoptosis by upregulating macroautophagy, which can facilitate cell survival during stress [47]. In quiescent GIST cells, apoptosis sensitivity could be restored by RNA interference-mediated (RNAi-mediated) knockdown of key autophagy regulators and by the antimalarial lysosomotrophic agents chloroquine and quinacrine. Withdrawal from the cell cycle and consequent resistance to the proapoptotic action of RTK inhibition also involves the nuclear accumulation of the cyclin-dependent kinase inhibitor p27\textsuperscript{Kip1} due to APCCDH1 complex-mediated proteasomal degradation of the p27\textsuperscript{Kip1}-regulator SKP2 [57]. Another recent study identified a role for the DREAM complex (DP, p130/RBL2, E2F4, and MuvB) in GIST cell quiescence induced by imatinib treatment [58]. Importantly, the authors found that quiescence could be abrogated and apoptosis sensitivity restored by the inhibition of dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), which plays an essential role in DREAM complex formation. Together, these results suggest that GIST could be sensitized to RTK inhibition-induced apoptosis by pharmacological inhibition of several cell-autonomous mechanisms that underlie cell cycle exit or stimulation of differentiation of Kit\textsuperscript{low/−} precursors.

The effects of these interventions may be limited by changes other than TP53 inactivation. We recently reported that in both imatinib-sensitive and imatinib-resistant GIST cells, and in tumorigenic ICC-SCs, reduced apoptosis sensitivity could also reflect loss of family with sequence similarity 96, member A (FAM96A) [59]. FAM96A is a member of the cytosolic iron-sulfur protein assembly machinery and a regulator of cellular iron homeostasis [60], but we found its proapoptotic action to bind apoptotic peptidase activating factor 1 (APAF1). Although FAM96A was robustly expressed in human and mouse ICCs, murine ICC-SCs, and PDGFRα-expressing interstitial cells, FAM96A protein or mRNA was dramatically reduced in human mouse ICCs, murine ICC-SCs, and PDGFRα-expressing interstitial cells, FAM96A protein or mRNA was dramatically reduced or lost in 106 of 108 GIST samples due to genomic deletion or other causes. Re-expression of FAM96A in GIST cells and transformed ICC-SCs increased apoptosis sensitivity and diminished tumorigenicity; however, it is unclear whether pharmacological sensitization to mitochondrial apoptosis (e.g., by TP53-modulating agents [24]) could overcome the effects of FAM96A loss.
MICROENVIRONMENTAL MECHANISMS OF GIST PERSISTENCE

GIST cells not dependent on constitutively active RTK signaling must draw on alternative, ligand-activated signaling pathways for survival. Pharmacological targeting of these mechanisms may provide additional means to eliminate cells contributing to disease persistence and to treat SDHB-deficient, RTK wild-type GISTs, which respond very poorly to imatinib and only slightly better to sunitinib [27, 61]. IGF1R is variably expressed and activated in several molecular subtypes of GIST [31]. Most robust expression has been observed in pediatric and pediatric-like adult GISTs [27–31, 61] reflecting either genomic amplification [31] or constitutive high expression [29, 30]. The latter may signify the less differentiated nature of these tumors and their possible relationship to ICC-SCs [27, 61], which, unlike mature ICCs, strongly express both Igf1r and insulin receptor (Insr) in possible relationship to ICC-SCs [27, 61], which, unlike mature ICCs, strongly express both Igf1r and insulin receptor (Insr) in possible relationship to ICC-SCs [27, 61], which, unlike mature ICCs, strongly express both Igf1r and insulin receptor (Insr) in possible relationship to ICC-SCs [27, 61].

The depicted mechanisms are not mutually exclusive. Abbreviations: GIST, gastrointestinal stromal tumor; WT, wild type.

CONCLUSION

Disease persistence in GIST involves multiple mechanisms including activation of signaling pathways triggering the cells’ exit from the cell cycle, autophagy, loss of proapoptotic proteins, downregulation of KIT/PDGFRα expression, or selection of GIST stem cells that do not depend on KIT/PDGFRα signaling for survival due to expression of alternative wild-type receptor tyrosine kinases (Fig. 2). In view of the diversity of the gene expression profiles of GISTs exposed to long-term imatinib treatment, eradication of residual tumor cells and cure of GIST will likely require individualized combinations of several approaches tailored to tumor genotype and phenotype.

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AUTHOR CONTRIBUTIONS

T.O.: conception and design, financial support, manuscript writing; M.Z.: manuscript writing, final approval of manuscript; Y.H.: assembly of data, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.
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