Biology and Genetic Aspects of Gastrointestinal Stromal Tumors: KIT Activation and Cytogenetic Alterations

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Recent studies have done much to reveal the biological and genetic underpinnings of gastrointestinal stromal tumors (GISTs). Constitutive activation of the KIT receptor tyrosine kinase is a central pathogenetic event in most GISTs and generally results from oncogenic point mutations which can involve either extracellular or cytoplasmic domains of the receptor. Oncogenic mutations enable the KIT receptor to phosphorylate various substrate proteins, leading to activation of signal transduction cascades which regulate cell proliferation, apoptosis, chemotaxis, and adhesion. KIT mutations can be broadly assigned to 2 groups, those that involve the "regulatory" regions responsible for modulating KIT enzymatic activity and those that involve the enzymatic region itself. In vitro studies suggest that GISTs with regulatory-region KIT mutations are more likely to respond to STI-571 than are GISTs with enzymatic-region mutations. A minority of GISTs lack demonstrable KIT mutations, but KIT is nonetheless strongly activated. Such GISTs might contain KIT mutations which are not readily detected by conventional screening methods, or alternately, KIT might be activated by nonmutational mechanisms. Most GISTs have noncomplex cytogenetic profiles, often featuring deletions of chromosomes 14 and 22. Additional chromosomal aberrations are acquired as the GISTs progress to higher histologic grade. These cytogenetic aberrations are undoubtedly important in GIST pathogenesis, but currently they do not play a key role as diagnostic adjuncts. HUM PATHOL 33:484-495. Copyright 2002, Elsevier Science (USA). All rights reserved.

Key words: **sarcoma, gastrointestinal neoplasm, receptor tyrosine kinase, mutation, KIT, oncogenic, cytogenetics**

Abbreviations: **FISH, fluorescent in situ hybridization; GIST, gastrointestinal stromal tumor; ICC, interstitial cells of Cajal; PCR, polymerase chain reaction; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase.**

KIT is a transmembrane tyrosine kinase receptor in which the extracellular portion binds a ligand known as stem-cell factor (also known as Steel factor)[1-3] and the intracellular portion contains the actual kinase enzymatic domain (Fig 1). KIT is similar in structure to several other receptor tyrosine kinases (RTKs) with oncogenic capabilities, including platelet-derived growth factor receptors (PDGFRs) A and B, CSF1R, and FLT3.[1,2] KIT activation normally occurs when two adjacent receptors are brought together through binding to ligand dimmers (Fig 1).[2,4,5] This process, known as homodimerization, is accompanied by structural changes in the receptors, resulting in activation of the KIT kinase domain. The activated kinases then crossphosphorylate tyrosine residues in the opposed homodimer partner, leading to additional KIT structural alterations and further activation of the receptor. The phosphotyrosines also serve as binding sites for various cell-signaling proteins, many of which are phosphorylated by KIT or by each other. These steps culminate in activation of intricate cell-signaling cascades that control crucial cell functions in tumorigenesis, including proliferation, adhesion, apoptosis, and differentiation (Fig 2).

KIT is expressed at high levels in hematopoietic stem cells, mast cells, melanocytic cells, germ cells, and the interstitial cells of Cajal (ICC).[6-9] The ICC comprise a complex network of innervated cells located between peripheral nervous system elements and smooth muscle cells in the wall of the gastrointestinal tract, and providing communication—particularly by regulating peristalsis—between nerve and smooth muscle.[8] The ICC appear to arise from uncommitted mesenchymal cells, which can differentiate to either mature ICC or smooth muscle.[8,10] Notably, KIT expression plays a crucial role embryologically in encouraging differentiation of primitive mesenchymal progenitor cells towards an ICC endpoint.[8,10,11] Moreover, KIT expression is requisite for proliferation of the committed ICC precursors, and KIT's role in ICC differentiation and proliferation is essential to the formation of a functional ICC network.[8-12] Accordingly, disruption of KIT (e.g., in mouse models) results in absence of a functional ICC compartment, as manifested by aperistalsis of the gut. These findings provide a developmental and biological basis for the ICC lineage hyperplasia—and eventual progression to neoplastic GIST—resulting from constitutive KIT activation.

KIT ACTIVATION: A CENTRAL TUMORIGENIC EVENT IN GISTs

Many GISTs express constitutively activated KIT oncoproteins. The oncogenic activation of these KIT

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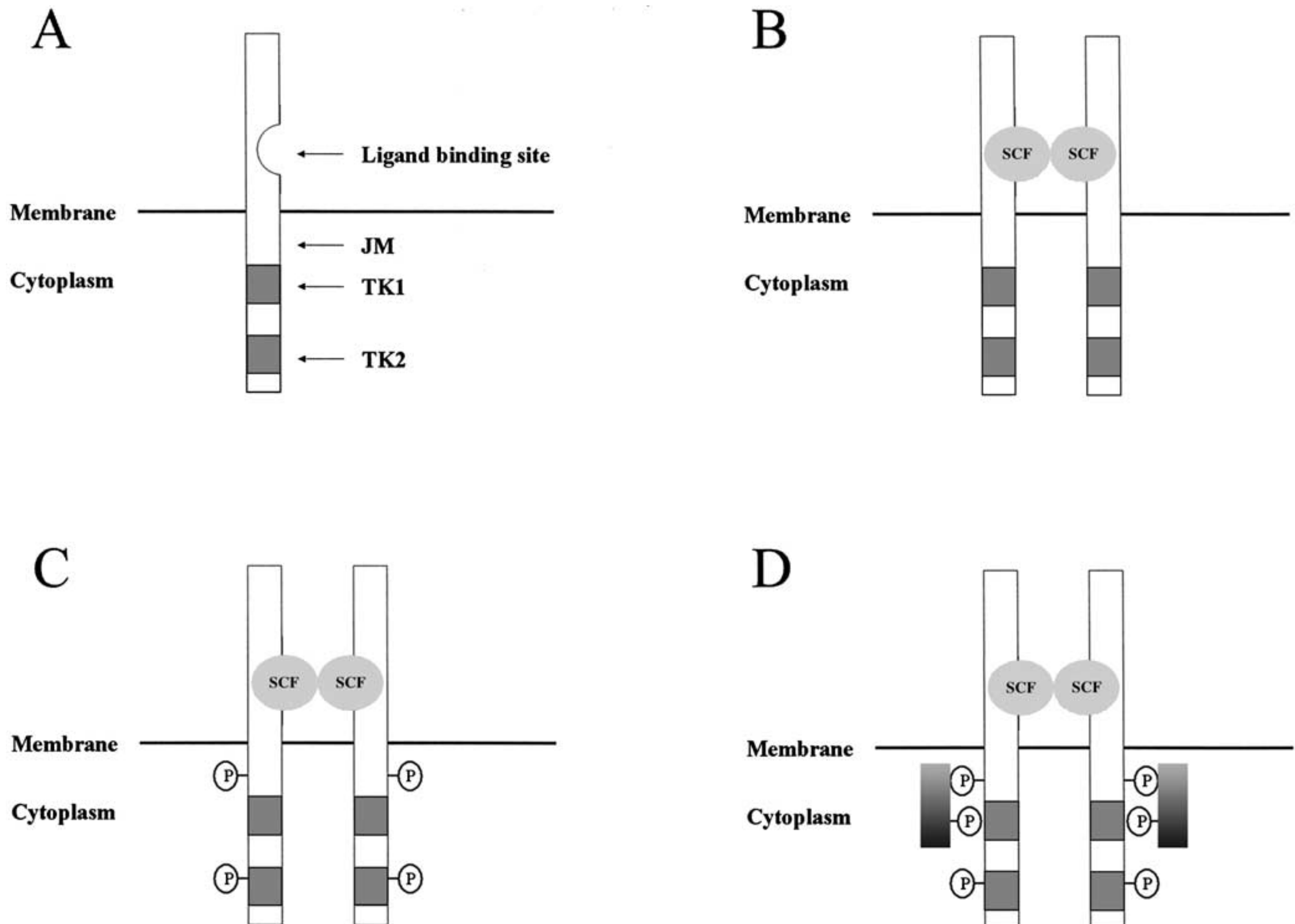

FIGURE 1. Ligand-induced activation of KIT tyrosine kinase activity. (A) Native KIT is expressed as a transmembrane protein. The cytoplasmic domain contains the tyrosine kinase portion of the protein (split into 2 domains, TK1 and TK2, as indicated). An *arrow* indicates the location of the juxtamembrane domain (JM). (B) Binding of dimeric ligand stem-cell factor (SCF), results in physical interaction of two KIT proteins. (C) Ligand-induced dimerization activates the cytoplasmic tyrosine kinase domains, resulting in autophosphorylation (P) of selected tyrosine residues. (D) Substrates and adapter proteins bind to phosphorylated tyrosine residues and in some cases are directly phosphorylated by KIT.

proteins does not depend on binding of KIT ligand; rather, KIT oncoproteins in GISTs often have structural changes which favor receptor oligomerization and cross-phosphorylation, even in the absence of ligand binding (Fig 3).[13-15] The activating mechanism in most GISTs is oncogenic mutation of the *KIT* gene itself, but there is evidence for alternate activating mechanisms in a subset of tumors. Expression of KIT oncoproteins is undoubtedly a pivotal event in GIST tumorigenesis, and KIT activation might be one of the earliest transforming events in GISTs. Indeed, KIT-activating mutations are the clear initiating events in some familial GISTs. Of note, although the germ line KIT mutations in familial GISTs support a central role for KIT activation, they also show that distinct KIT activation mechanisms are found in different GIST syndromes and perhaps determine the phenotype in each syndrome.[16-19] For example, inheritance of activating juxtamembrane region KIT mutations is associated with ICC hyperplasia, GISTs, cutaneous mastocytosis (urticaria pigmentosa), and/or cutaneous hyperpigmentation.[16,18-20] On the other hand, inheritance of an activating mutation of the KIT kinase domain (K642E) is associated with ICC hyperplasia and multiple GISTs, but not with mastocytosis or cutaneous hyperpigmentation.[17] Therefore, both juxtamembrane and kinase region KIT mutations provide proliferative stimulus to GIST progenitor cells, but only the juxtamembrane mutations provide proliferative signals to mast cell and melanocyte precursors. The GISTs in these syndromic individuals, irrespective of underlying KIT mutation type, are often not apparent clinically until middle age. This suggests that KIT activation is sufficient for ICC hyperplasia, whereas successive, additional oncogenic events, perhaps involving genes other than KIT, are needed to create localized, discrete, and truly neoplastic GISTs. Many of the recurring KIT mutations in GISTs have not been reported in other malignancies (i.e., germ cell tumors, mast cell disease, myelofibrosis, chronic myelogenous leukemia) that feature KIT mutations.[13,14,21-26] Likewise, only a subset of the known KIT oncogenic mutations appear to confer proliferative

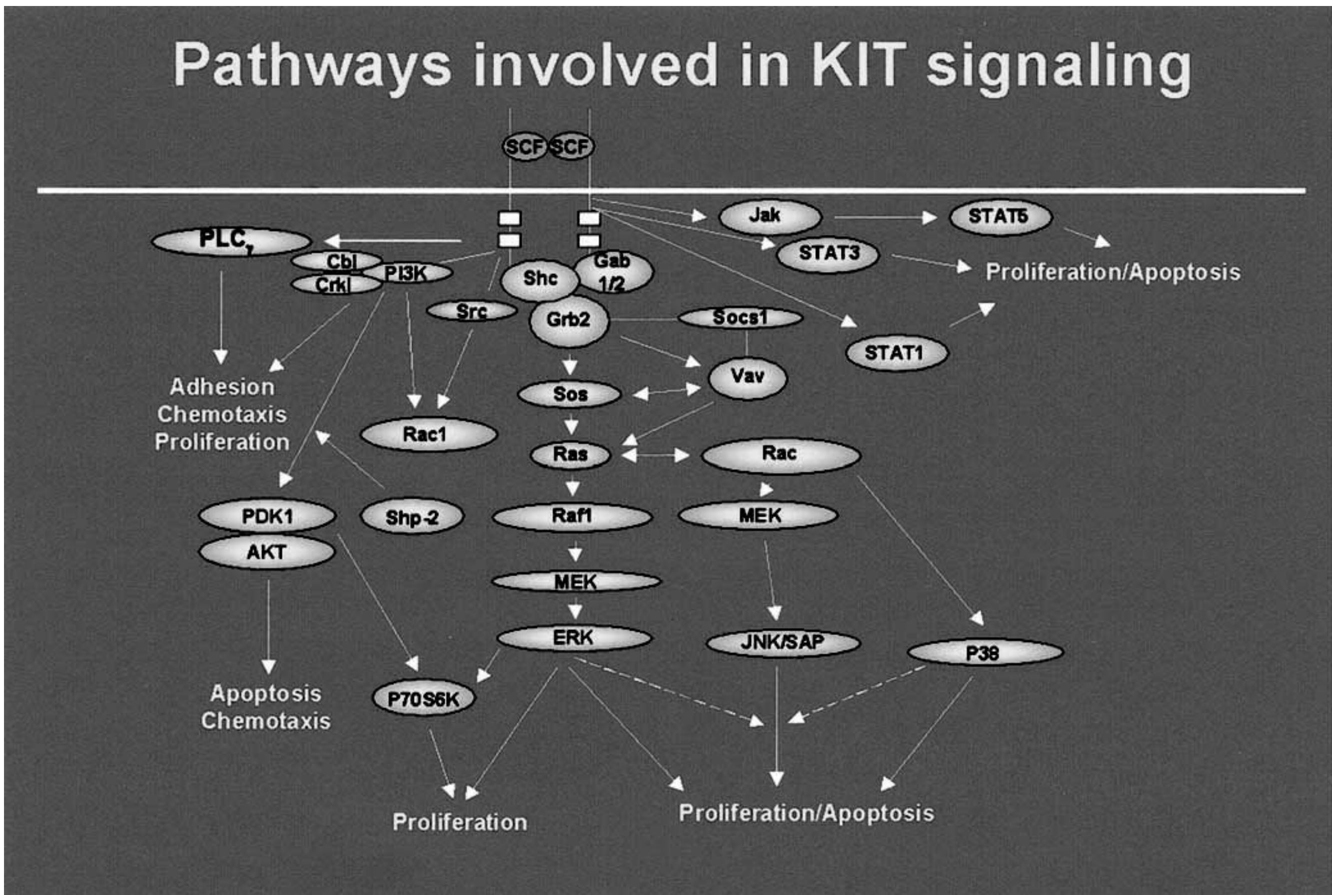

FIGURE 2. Cell-signaling cascades resulting from KIT receptor tyrosine kinase activation. This schematic is a simplified representation of the known signaling pathways activated by KIT.

advantage in GIST precursor cells. For example, KIT mutations involving the critical amino acid residue D816 are common in mast cell disease[27] but have not been found among more than 500 GISTs. Thus D816 substitutions, although pivotal in mast cell hyperplasia, can be assumed to be relatively ineffective in accomplishing transformation of the GIST progenitor cell.

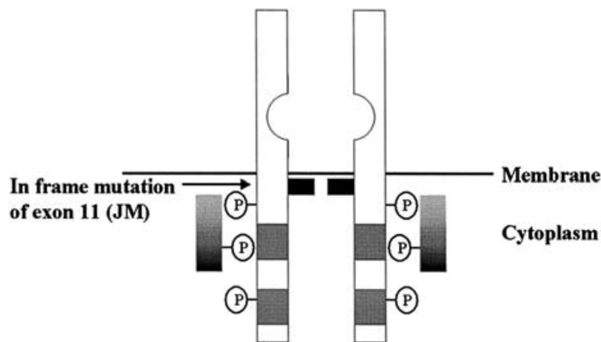

FIGURE 3. Oncogenic mutations of the KIT juxtamembrane domain result in ligand-independent KIT dimerization and activation of the kinase enzymatic domain. KIT activation is followed by receptor autophosphorylation and biding of substrate and adapter proteins. In some cases KIT directly phosphorylates these KIT-associated proteins.

Our unpublished studies (J.A.Fletcher et al, personal communication) suggest that KIT mutations are acquired before cytogenetic aberrations during GIST tumorigenesis. Several observations support this view. It is not unusual to encounter GISTs in which the characteristic cytogenetic aberrations (e.g., deletions of 14q and 22q) are found in only a subset of the neoplastic cells when evaluated by karyotyping and interphase fluorescent in situ hybridization (FISH). However, sequencing correlations suggest that *KIT* mutations are present in nearly all of the neoplastic cells in most GISTs. In the same vein, karyotyping and molecular cytogenetic analyses show that some GISTs have entirely normal (diploid) cytogenetic profiles, despite the demonstrated presence of activating KIT mutations. These studies are consistent with an early—and potentially initiating— role for KIT activation in GIST tumorigenesis, whereas the characteristic cytogenetic aberrations are likely involved in neoplastic progression.

Although KIT activation is a predominant oncogenic mechanism in GISTs, it is possible that other, as-yet undefined transforming mechanisms are of similar importance. It is also possible that oncogenic activation of KIT downstream signaling intermediates can supplant KIT activation in some GISTs. Such mechanisms have been observed in several in vitro and mu-

rine GIST models, in which KIT expression is silenced despite continued rapid cell proliferation and high-level activation of KIT downstream signaling proteins (M. Heinrich and J. Fletcher, unpublished observations).

Despite general agreement that KIT activation plays a critical oncogenic role in GISTs, there is no consensus as to the frequency of *KIT* mutations. Various studies, all involving polymerase chain reaction (PCR) sequencing analyses of 35 or more tumors, have found *KIT* genomic mutations in anywhere from 21% to 92% of GISTs.[15,28-31] Several factors might contribute to these extremely discrepant results. First, every series in which *KIT* mutations were found in less than 50% of GISTs, evaluated only a segment of the mutational "hot-spot" region in exon 11 (i.e., the region encoding part of the KIT juxtamembrane region).[28-30] Activating mutations in 3 other *KIT* domains, which contain a substantial number of the overall mutations, were not evaluated. Second, the *KIT* exon 11 PCR assays in several series did not evaluate the 3' end of the juxtamembrane region,[28-30] which is mutated by duplication events in a substantial minority of GISTs, including those of lower histologic grade (J.A. Fletcher and B.P. Rubin, unpublished data). Third, genetic differences in the study populations, or differences in the biology of the GISTs in each series, might contribute to the apparent variation in *KIT* mutational frequency. Notwithstanding, it is our experience that systematic sequencing of the juxtamembrane coding region, coupled with evaluation of the entire *KIT* coding sequence in GISTs that lack juxtamembrane region mutations, reveals oncogenic KIT mutations in most GISTs, irrespective of histologic grade (Fig 4).[15;32] In addition, most GISTs have constitutive biochemical activation of the KIT protein, manifested by high-level tyrosine phosphorylation, irrespective of their histologic grade.[15]

These observations, together with the instrumental roles played by KIT in ICC developmental biology and familial GISTs, support a crucial and early need for KIT oncogenic activation in the development of benign and malignant GISTs. As discussed before, we favor a model in which KIT oncogenic activation often occurs in the very earliest stages of benign GIST formation, with successive cytogenetic and molecular mutations then driving progression to more malignant behavior.

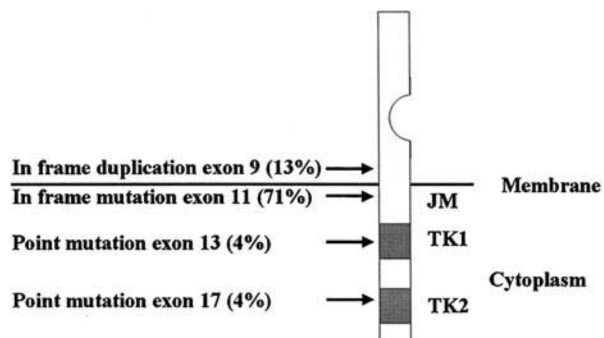

FIGURE 4. Oncogenic KIT mutations involve the extracellular, juxtamembrane, and kinase domains. Rubin et al.[15] reported oncogenic KIT mutations in 44 of 48 GISTs; 71% of GISTs had juxtamembrane region mutations, whereras another 21% had mutations outside this region.

MECHANISMS OF KIT REGULATION AND DYSREGULATION

As is the case with most RTKs, KIT kinase activity has multiple levels of control. Disruption of any of several control mechanisms may lead to aberrant kinase activation and oncogenesis. KIT kinase activity is controlled first and foremost by developmentally specific expression of the KIT protein. (If there is no protein expression, then there is no kinase activity). In the absence of ligand, normal KIT exists as a monomer in which enzymatic activity is inhibited by intrinsic structural component(s).[33] KIT autoinhibition is another level of control beyond cell-specific expression mechanisms. Autoinhibition prevents constitutive KIT activation and allows for specific responses to externally produced ligands. Normal activation occurs when bivalent stem-cell factor dimers interact with KIT, resulting in autophosphorylation. The autophosphorylation of particular tyrosine residues antagonizes autoinhibitory structural conformations and thereby leads to kinase activation, which results in further phosphorylation of KIT and "downstream" receptor substrates. The effects of activation may be modified by substrate availability and binding and by phosphatase-mediated dephosphorylation of activated KIT.

To better describe the events resulting in dysregulation, it is useful to divide the mechanisms of control of kinase activity into those that are extrinsic to *KIT* (e.g., the presence of extracellular ligand or the intracellular levels of substrate and phosphatase molecules), and those that are intrinsic to *KIT* and thus can be modified by *KIT* mutations. Mutations resulting in the loss of kinase function do not appear to be involved in the pathogenesis of GISTs. KIT-activating mutations, as found in most GISTs, can be conceptualized as belonging to 1 of 2 groups.[34,35] The first group comprises mutations that alter the amino acid sequence of regions forming the active kinase "pocket," thus directly affecting the primary and higher-order structures of the enzymatic site. These types of mutations can be called "enzymatic pocket"– or "enzymatic site"–type mutations and are epitomized by D816V substitution, a characteristic mutation in adult human mastocytosis. The D816V mutation affects the activation loop at the entrance to the enzymatic pocket formed by the split intracellular kinase domain of KIT.[27,34,36] The second group of mutations involve regulatory portions of the KIT protein, particularly the regions with autoinhibitory function. These mutations, termed "regulatory-type" mutations, differ from enzymatic site–type mutations in that they preserve the normal amino acid sequence of the enzymatic site.

The distinction between regulatory and enzymatic site mutation mechanisms is important clinically, because KIT inhibition by some small molecule com-

pounds depends in part on whether KIT is activated by regulatory or enzymatic site mutations.[34,37] In particular, mutations of amino acids that participate directly in binding to therapeutic inhibitors (e.g., in the KIT enzymatic site) often render the inhibitors totally ineffective.[34,35,37] Thus, by identifying regions of KIT affected by mutations in specific tumors, it might be possible to predict the response of mutant KIT to various inhibitors, and thereby guide selection of optimal drugs for treatment of individual patients.

Regulatory-type *KIT* mutations are found in most GISTs.[15] The best-characterized KIT regulatory region is the intracellular juxtamembrane region, encoded by exon 11. The secondary structure of this region includes an amphipathic alpha helix that suppresses KIT phosphorylation and kinase activity.[33] Mutations that disrupt the alpha helix release the inhibitory effects of the juxtamembrane region, resulting in KIT gain of function (i.e., constitutive activation). Interestingly, a similar juxtamembrane autoinhibitory helix was recently identified in the EphB2 RTK and was shown to function through direct interaction with the EphB2 kinase domain.[38] Thus kinase autoinhibition via juxtamembrane secondary structures, such as alpha helices, is a generalizable mechanism of kinase regulation in different RTK families. The types of mutations that perturb the autoinhibitory function of the KIT juxtamembrane helix include those that introduce hydrophilic amino acids into the hydrophobic portion of the helix; those that substitute amino acids, such as proline, that impose structural constraints incompatible with helix formation; and those that destroy the helix by causing major alterations of the primary amino acid sequence (e.g., by large deletions or insertions).[38] Thus a broad range of mutational events affecting this region can disrupt the autoinhibitory alpha helix and result in constitutive KIT activation. This paradigm explains why diverse juxtamembrane mutations are characteristic of GISTs[28] and other types of neoplasia.[39] A key feature of these juxtamembrane region regulatory-type mutations is that the KIT oncoproteins have essentially the same active enzymatic site as that in native KIT. Therefore, kinase inhibitors such as STI-571, which bind well to the enzymatic site of native KIT, are also effective inhibitors of these constitutively activated molecules.

The extracellular ligand-binding domain of KIT is another region involved by oncogenic mutations, as reported in GISTs,[32,40,41] myeloproliferative disorders,[25,42] and acute myelogenous leukemia.[43] Extracellular region mutations of KIT are activating in nature,[15,41] and their inhibition profile is compatible with a regulatory type function; that is, they are sensitive to kinase inhibitors at concentrations that inhibit native KIT (B.J. Longley, Y. Ma, J.A. Fletcher, and M.C. Heinrich, unpublished observations). In contrast to regulatory-type mutations, enzymatic site–type mutations, which activate KIT by altering the primary sequence of the enzymatic site, may have unpredictable effects and often result in resistance to inhibitors that are effective against wild-type KIT.[35] For instance, the KIT codon 816 substitutions found in some forms of human mastocytosis are predicted to alter the charge of the mobile activation loop at the entrance to the active kinase site. As a group, these activating mutations are resistant to STI-571 and other clinically proven compounds that competitively inhibit ATP use by wild-type KIT (B.J. Longley and Y. Ma, unpublished observations).[35] Activating mutations that alter amino acids predicted to help form the enzymatic pocket have also been found in occasional GISTs. Some of these appear to be sensitive to STI-571 in vitro (M.C. Heinrich, unpublished observations), whereas others are resistant to this drug (B.J. Longley, unpublished observations).

It appears likely that activating mutations affecting other enzymes may also be classified as "regulatory" or "enzymatic site" in type, and that this paradigm may prove generally useful in predicting drug resistance and guiding therapy. In this vein, a recent report by Gorre et al.[44] described diverse mechanisms of STI-571 resistance in 9 patients with *BCR-ABL*–positive CML. STI-571 resistance was associated with acquired mutations of the ABL enzymatic site in 6 patients and with *BCR-ABL* gene amplification in the other 3. Therefore, resistance was due to either enzymatic site–type mutations or, in the case of the gene amplifications, to the defeat of STI-571 inhibition via overexpression of the intrinsically sensitive regulatory-type mutations. Gorre et al suggested that the patients with gene amplification might be susceptible to treatment with higher doses of STI-571, whereas those with active site mutations may require treatment with a different drug.

BIOLOGICAL CONSEQUENCES OF KIT ACTIVATION IN GISTs

KIT activation normally occurs when the receptor is bound by its cognate ligand, stem-cell factor. Ligand-mediated KIT activation triggers various cell-signaling cascades that regulate cell behavior.[45,46] Examples of critical downstream signaling mechanisms include activation of cell survival (antiapoptotic) proteins, such as AKT, and cell proliferation–related proteins, such as MAPK p42/p44.[45;46] An intriguing aspect of KIT oncoproteins in hematologic neoplasms is that the exact signaling pathways activated by the mutant KIT differ from those activated by normal KIT.[47,48] We believe that this is also the case in GISTs, where the signaling cascades governed by KIT oncogenic activation do not necessarily coincide with those resulting from ligand-mediated activation of the normal KIT receptor (M.C. Heinrich, A. Duensing, and J.A. Fletcher, unpublished studies). For example, ligand-mediated KIT activation generally leads to phosphorylation of the AKT and MAPK (p42/44) signaling intermediates, which have antiapoptotic and proliferation-inducing roles. In contrast, AKT and MAPK phosphorylation are sometimes inconspicuous in GISTs expressing constitutively activated, oncogenic KIT proteins (Fig 5). The same holds for other KIT pathway signaling proteins, including STAT1 and STAT3, for which phosphorylation can vary depending on the KIT mutation mechanism. Thus KIT

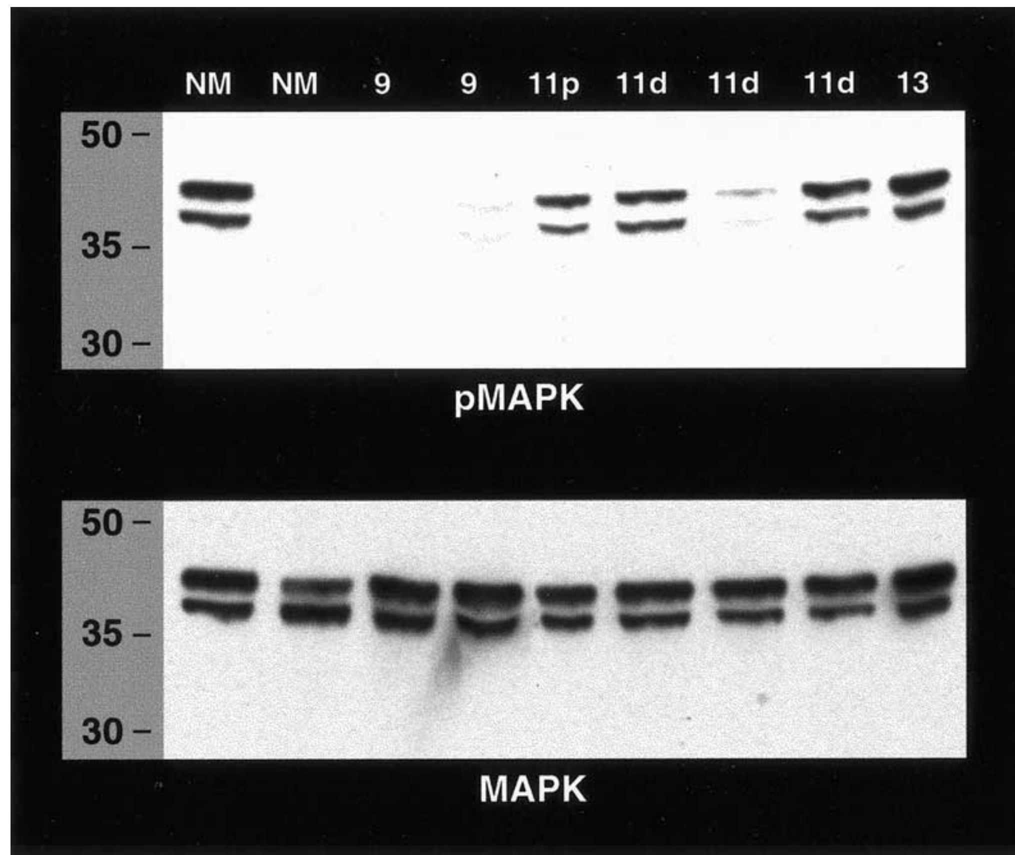

FIGURE 5. Variable activation of MAPK p42/44 in GISTs. Primary GIST cell lysates were western blotted and immunostained, sequentially, for phosphoMAPK p42/44 (A) and total MAPK (B). The lane headings NM, 9, 11p, 11d, and 13 denote GISTs with no demonstrable KIT mutation, exon 9 mutation, exon 11 point mutation, exon 11 deletion mutation, and exon 13 mutation, respectively. MAPK phosphorylation is variable, being weakest in the 2 GISTs with exon 9 mutation (lanes 3 and 4), and in 1 of the GISTs with wild-type KIT (lane 2).

oncogenic mutations are highly selective in the downstream pathways that they activate, and the biological consequences of KIT oncogenic mutation are expected to differ from those resulting from ligand-mediated activation of the normal KIT receptor.

As discussed earlier, we have inklings as to which GIST cell signaling pathways are activated by different categories of mutant KIT oncoproteins. At this time, however, there are no published data to pinpoint which signaling pathways are requisite for KIT transforming activity in GISTs. In addition, the biological consequences of oncogenic KIT signaling have not been determined in GISTs. Considerable work is needed to address these important questions. Certain proteins, including members of the STAT family,[45,46,49] are believed to serve critical roles in KIT oncogenic signaling in non-GIST tumor models. These models are useful starting points in the evaluation of KIT mechanisms in GISTs; however, they cannot be relied on to pinpoint the crucial transforming pathways in GISTs. This is because many KIT cell signaling proteins have varying biological roles, depending on mechanisms, timing, and cell context of activation. For example, activated STAT1 has oncogenic functions in some cancers but is proapoptotic in others.[50] Likewise, activated JNK can be predominantly mitogenic/transforming or proapoptic, depending on cell context and the signaling pathways responsible for its activation.[51,52] Thus the KIT signaling data in various normal and non-GIST cancer cell models do not predict which signaling pathways are critical for KIT oncogenic behavior in GISTs. The available models (some of which involve RTKs closely related to KIT, such as PDGFR-B and PDGFR-A) support 4 hypotheses:[53-57]

1. Signaling pathways activated by KIT oncoproteins modify crucial aspects of the GIST cell phenotype, including differentiation, adhesion, apoptotic activity, and mitogenic activity.
2. The GIST signaling pathways that are activated by oncogenic KIT differ from those in nonneoplastic cells.
3. The exact signaling pathways activated by KIT oncoproteins vary from GIST to GIST, depending on the location and nature of the KIT mutation.
4. Critical clinicopathologic and biological parameters, including GIST apoptotic activity, mitotic activity, metastatic capability, and STI-571 response, are influenced by KIT sequence and structure.

Elucidation of the oncogenic KIT signaling pathways, particularly those that are crucial in transmitting the oncogenic signal, is a matter of substantial clinical importance. It is expected that some GISTs will ultimately become resistant to KIT inhibitor therapies. Such resistance might develop if the KIT oncoprotein acquires new mutations, or if other proteins in the KIT signaling pathways acquire mutations, rendering the upstream KIT signal irrelevant. Thus essential signaling proteins must be identified that can serve as additional

therapeutic targets to more effectively silence KIT signaling pathways in GISTs.

ALTERNATE KIT ACTIVATION MECHANISMS IN GISTs

KIT signaling pathways are activated in virtually all GISTs, and most GISTs have oncogenic mutations within the coding sequence of the *KIT* gene. However, even those tumors that lack such mutations can have biochemical features of high-level KIT kinase activation.[15] As discussed later, KIT might be activated oncogenically by several alternate, nonmutational mechanisms, but to our knowledge, none of these mechanisms has been tested rigorously. It is important to consider alternative (i.e., non-GIST) diagnoses in tumors that lack apparent KIT mutations, particularly when the histology is atypical and when KIT immunohistochemical staining is either weak or focal. Another possibility, in GISTs lacking apparent *KIT* mutation, is that the DNA or RNA template used for mutational analysis is derived largely from nonneoplastic cells. This scenario is particularly likely when the biopsy specimen is necrotic, heavily infiltrated by nonneoplastic cells, or composed of either tumor capsule or other peritumoral nonneoplastic tissue. Thus it is appropriate to consider microdissection of paraffin-embedded neoplastic cells from *KIT* wild-type GISTs to prove that these truly lack mutations. Some 90% of GISTs are heterozygous for the *KIT* mutation, containing 1 normal and 1 mutated allele, and a small amount of admixed nonneoplastic tissue can readily obscure the mutation when analyzed by conventional genomic screening methods. Our data (B.P. Rubin and J.A. Fletcher, unpublished data) suggest that *KIT* mutations can be missed by routine screening methods, such as automated sequencing of genomic PCR products, when neoplastic cells compose less than 50% of the tissue sampled. However, we have found that high-pressure liquid chromatography–based screening strategies can detect mutant *KIT* alleles even when admixed with substantial amounts of wild-type *KIT* from nonneoplastic cells (M.A. Heinrich, unpublished data).

In addition to tumors that have been misclassified, or in which *KIT* mutations have been missed due to technical considerations, there are undoubtedly GISTs in which alternate mechanisms of activation account for the absence of *KIT* mutations. Such mechanisms might include genomic alterations in noncoding regions of the *KIT* gene, or alteration in the expression or function of proteins known to modulate the KIT cell signaling pathways. *KIT* mutations outside of the coding sequences might involve the regulatory regions of the *KIT* gene that control transcription or those regions involved in splicing. Mutations or altered methylation in promoter regions could lead to substantial up-regulation of *KIT* transcription, whereas mutations of intronic splice site motifs could lead to mutant KIT proteins with heightened function. Alternatively, genomic amplification of the *KIT* locus could lead to KIT overexpression and activation of the KIT pathway.

Another potential mechanism of KIT activation is aberrant expression or altered activity of proteins that interact with KIT. KIT participates in complex networks of signal cascade proteins (see Fig 2), and some of these proteins regulate KIT activation in a positive or negative manner. KIT pathway activation could result from mechanisms that target KIT directly, such as autocrine/paracrine KIT stimulation by ligand (stem-cell factor), by heterodimerization with activated RTKs that are structurally related to KIT, or by inactivation of phosphatases that inhibit KIT tyrosine phosphorylation.[47,58,59] Alternately, KIT signaling pathways might be activated by oncogenic events targeting proteins further down in those pathways, such as Ras.

It is also possible that a shift in the usual balance of KIT isoforms could predispose to neoplastic transformation. Four human KIT isoforms are expressed in both neoplastic and normal cells. These isoforms result from alternative splicing events and differ in the presence or absence of an extracellular region gycine-asparagine-asparagine-lysine sequence, encoded by exon 9,

TABLE 1. Characteristic Cytogenetic and Molecular Events in GISTs and in Mesenchymal Tumors That Can Masquerade as GISTs

Tumor Type	Characteristic Cytogenetic Events	Molecular Events
Gastrointestinal stromal tumor	Monosomies 14 and 22 Deletion of 1p	KIT mutation
Desmoid	Deletion of 5q Trisomies 8 and 20	APC mutation
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZFI-JJAZI fusion
Inflammatory myofibroblastic tumor	2p23 rearrangement	ALK fusion genes
Leiomyoma-uterine	t(12;14)(q15;q24) or Deletion of 7q	HMGIC rearrangement
Leiomyoma-extrauterine	Heterogeneous	
Leiomyosarcoma	Complex*	
Liposarcoma-dedifferentiated	Ring or giant marker chromosomes	
Schwannoma-malignant	Complex*	
Schwannoma-benign	Deletion of 22q	NF2 inactivation

*Indicates the consistent finding of extremely complex karyotypes, containing numerical and structural aberrations.

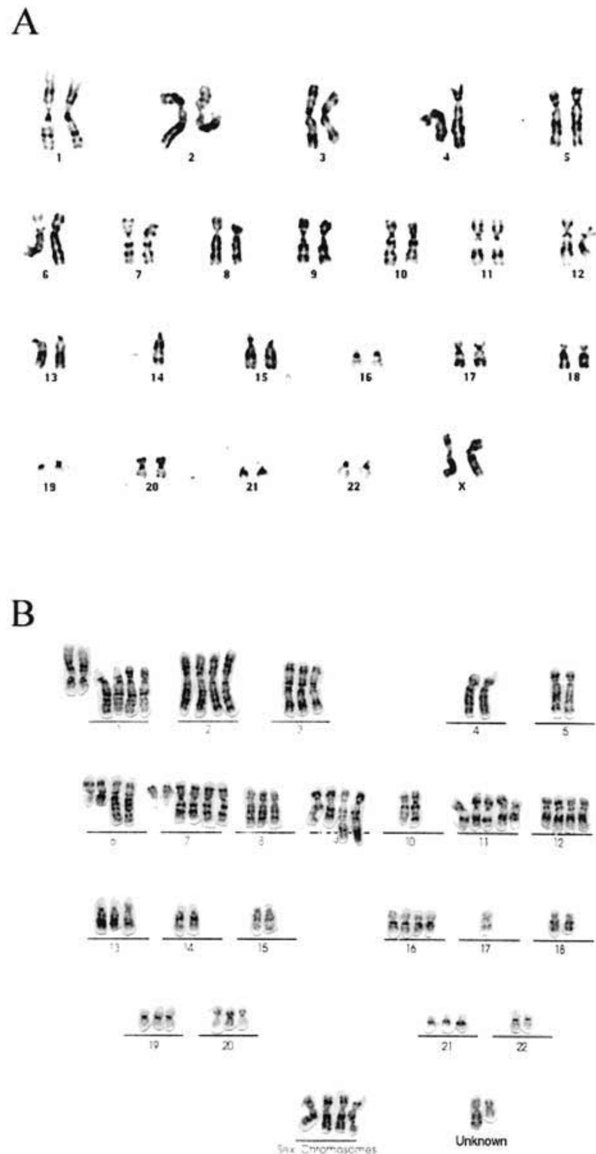

FIGURE 6. Comparison of cytogenetic complexity in GIST and leiomyosarcoma. GIST karyotype (A) is noncomplex, showing loss (monosomy) of chromosome 14 as a solitary chromosomal aberration. Leiomyosarcoma karyotype (B) is hyperdiploid with numerous chromosome gains, losses, and rearrangements. The tumors were of comparable histologic grade.

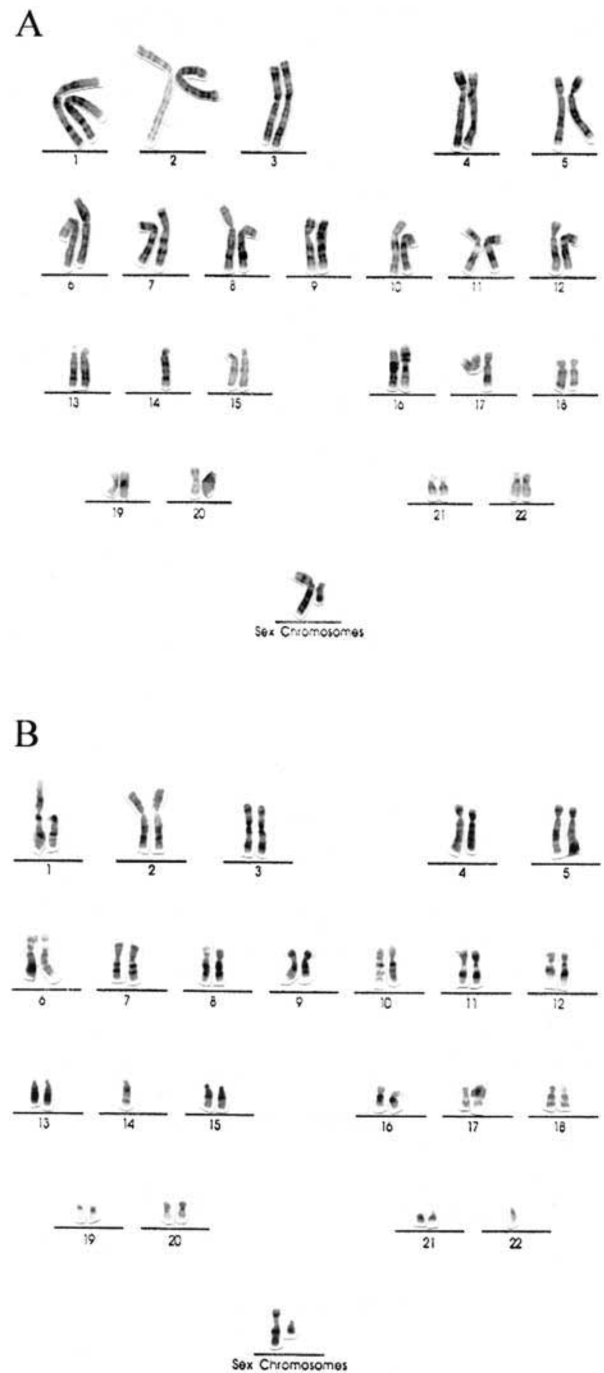

FIGURE 7. Cytogenetic progression in a GIST. Metaphase cell analysis reveals clonal cytogenetic progression, as is typically seen in malignant GISTs. Three metaphase cell subclones were identified, including those with monosomy 14 alone (A), those with monosomy 14 and monosomy 22 (not shown), and those with monosomy 14, monosomy 22, and 1p deletion (B). Therefore, the sequence of acquired chromosomal aberrations is monosomy 14 → monosomy 22 → deletion 1p.

and an interkinase region serine, encoded by exon 15.[60-62] Ashman et al[61] have reported that KIT lacking the extracellular gycine-asparagine-asparagine-lysine is the more tumorigenic isoform in nude mice xenografts, and thus it is possible that selective expression of this normal isoform might favor neoplastic transformation in some GISTs.

It is important to note that a small subgroup of GISTs are essentially KIT-negative, having low to undetectable KIT protein expression by immunohistochemistry and Western blotting evaluations. Preliminary studies suggest that such GISTs express alternative RTK oncoproteins that are structurally related to KIT (J.A. Fletcher and M.C. Heinrich, unpublished observations). Expression of KIT versus alternative RTK oncoproteins appears to be an "either/or" event in GISTs, with the alternative (non-KIT) mechanism being used in no more than 5% of cases.

CYTOGENETIC MECHANISMS IN THE PROGRESSION FROM LOW-GRADE TO HIGH-GRADE GIST

Most GISTs have 1 or more chromosomal deletions, and the cytogenetic profile in GISTs is quite different from that in histologic mimics such as leiomyoma and leiomyosarcoma (Table 1). The cytogenetic aberrations in a given GIST are typically found in only a subset of the neoplastic cells and thus can be viewed as "secondary" events, acquired after the oncogenic *KIT* mutations. Moreover, as discussed earlier, some GISTs have normal karyotypes but have *KIT* activating mutations. Therefore, it is possible that *KIT* point mutations initiate the neoplastic process in many GISTs, whereas cytogenetic aberrations are important in the biological and clinical progression of those tumors. The exact genes targeted by GIST cytogenetic aberrations remain to be identified, and whether *KIT* molecular mutations and cytogenetic aberrations work together in modifying specific GIST cell biological pathways is unclear. One intriguing possibility is that some GIST cytogenetic aberrations might alter signaling pathways downstream of the activated KIT receptor, and thereby heighten KIT transforming properties.

Most malignant GISTs have karyotypes that are far less complex than those in other spindle-cell tumors of comparable histologic grade (Fig 6). Indeed, the simplicity of most GIST karyotypes enables the cytogeneticist to track acquisition of various chromosomal deletions in individual tumors. However, the cytogenetic literature for GISTs is confusing, because karyotypes published before 1995 were generally described as being from GI leiomyomas and leiomyosarcomas. In retrospect, it is interesting to note that various cytogeneticists had reported a distinctive "subgroup" of GI leiomyosarcomas with noncomplex karyotypes and deletions of chromosomes 14, 22, and 1p.[63-66] These, of course, are exactly the tumors that are now known to be GISTs. Notably, a GIST—reported as small bowel leiomyosarcoma, but with the cytogenetic profile that we now know to be classic for GIST—was among the first 10 solid tumor karyotypes published.[67] Our single-institution experience in karyotyping 50 GISTs demonstrates that GISTs of varying histologic grades are on a cytogenetic continuum (J. Fletcher, unpublished data). Benign GISTs often have a normal karyotype or may have an isolated loss of chromosome 14. Borderline-malignant GISTs invariably have loss of chromosome 14, often accompanied by loss of 1p, 9p, 11p, or 22q Figs 7 and 8). High-grade malignant GISTs typically contain at least 3 of the aforementioned chromosomal deletions, although their karyotypes remain substantially less complex than those in most cancers that are histologic GIST mimics (e.g., high-grade malignant peripheral nerve sheath tumor, high-grade leiomyosarcoma, undifferentiated carcinoma). Notably, we are unaware of GISTs in which the KIT locus itself is targeted by chromosomal rearrangement (Fig 9). The absence of *KIT* cytogenetic rearrangements is consistent with the notion that KIT activation results from oncogenic mutations that bestow constitutive kinase activity on a protein that is already expressed at high levels in the GIST nonneoplastic progenitor cells. Molecular cytogenetic screening, particularly with the comparative genomic hybridization method, also re-

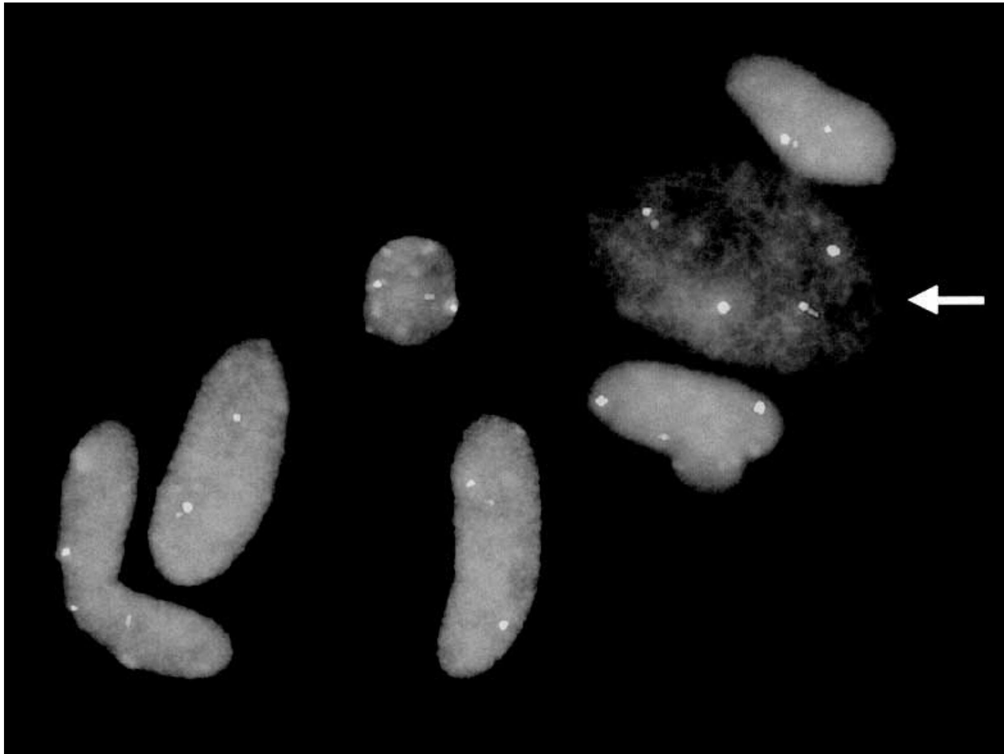

FIGURE 8. FISH demonstration of chromosome 14 and 22 deletion in interphase GIST cells. Fluorescein isothiocyanate signals (*green*) identify an alpha-satellite DNA sequence from the pericentromeric regions of chromosomes 14 and 22. Rhodamine signals (*red*) identify the *NF2* locus on the chromosome 22 long arm. A normal cell (*arrow*) has 4 green and 2 red signals. The remaining cells have a loss of 2 green signals and 1 red signal, indicative of monosomies 14 and 22.

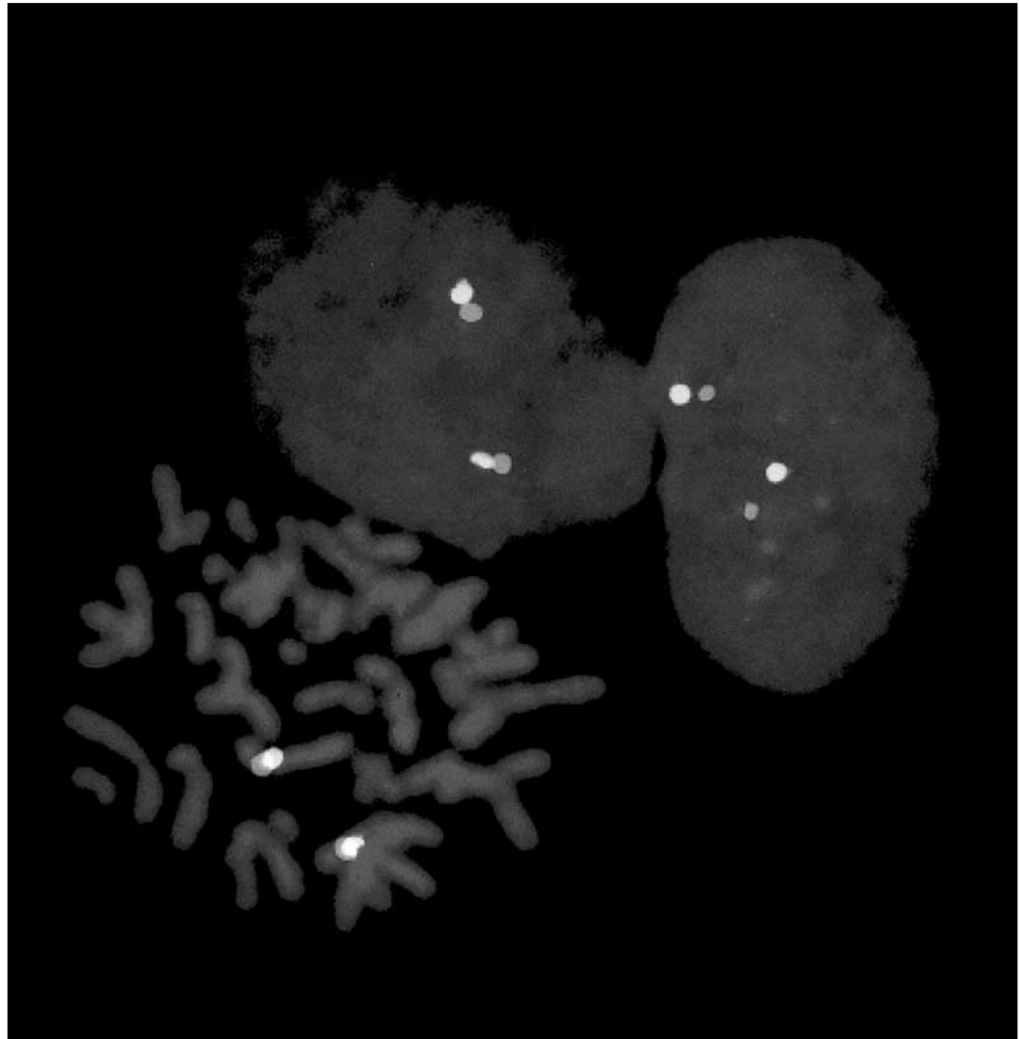

FIGURE 9. FISH evaluation of KIT locus rearrangement in a GIST. Fluorescein isothiocyanate signals (*green*) identify the KIT locus at chromosome band 4q12, and rhodamine signals (*red*) identify the adjacent chromosome 4 pericentromeric region. The KIT locus is nonamplified and nonrearranged in most GISTs.

veals compelling correlations between acquisition of chromosomal aberrations and aggressive clinicopathologic behavior. El-Rifai et al[68] reported such correlations in 95 GISTs, including 24 benign, 36 malignant primary, and 35 metastatic tumors. The mean number of demonstrable chromosomal aberrations was 2.6 in the benign GISTs, 7.5 in the malignant primary GISTs, and 9 in the metastatic GISTs. Deletions of chromosome arms 1p, 14q, and 22q were frequent findings in this GIST group, irrespective of histologic grade. However, other aberrations—including 9p deletion, 8q amplification, and 17q amplification—were found almost exclusively in high-grade (malignant) GISTs.[68]

To summarize, cytogenetic and molecular data indicate consistent pathogenic mechanisms for GIST clinical progression. A simplified version of the genetic progression is: KIT activating mutation → 14q deletion → 22q deletion → 1p deletion → 8q gain → 11p deletion → 9p deletion → 17q gain.

However, this description is a generalization, and not all GISTs acquire successive deletions in this order. This scenario is reminiscent of that in chronic myelogenous leukemia, wherein additional cytogenetic abnormalities above and beyond the *BCR-ABL* fusion are acquired during progression to accelerated phase and blast crisis.

SUMMARY

This review has outlined the considerable recent progress in our understanding of biological and genetic mechanisms in GISTs. Our intention has been to emphasize aspects of GIST "basic science" that are germane to the intriguing clinicopathologic features of GISTs. Our focus has been primarily on science and biological mechanisms rather than on clinical correlates. The relevance and application of these scientific advances, in the pathology and clinical fields, is discussed in several companion papers in this issue of HUMAN PATHOLOGY.

Constitutive activation of the KIT RTK is a critical event in the pathogenesis of most GISTs. KIT activation often results from oncogenic point mutations involving the KIT cytoplasmic juxtamembrane region (i.e., immediately adjacent to the transmembrane region). However, a minority of GISTs have mutations in other aspects of the KIT protein, including the extracellular

region and the enzymatic domain. In addition, a minority of GISTs lack demonstrable KIT mutations, even when the entire DNA coding sequence is examined. Interestingly, KIT is nonetheless highly activated in some of these mutation-negative tumors. The biological sequelae of KIT activation include KIT-mediated phosphorylation of various substrates, many of which also have intrinsic kinase functions, and resulting activation of signal transduction cascades. Through these cell-signaling pathways, the KIT oncogenic mechanism is translated into aberrant cell proliferation, apoptosis, chemotaxis, and adhesion. Notably, the location and nature of the KIT oncogenic mutations appear to influence the likelihood of clinical response to various KIT inhibitor therapies. In vitro and clinical studies for GISTs and other tumors with KIT mutations suggest that some KIT enzymatic "pocket" mutants are not effectively inhibited by STI-571. Fortunately, the KIT mutations in GISTs generally involve regulatory (i.e., nonenzymatic) regions of the receptor, and most such regulatory- region KIT mutants are intrinsically sensitive to STI-571. KIT mutation can be viewed as the initiating oncogenic event in syndromic GISTs from individuals with demonstrable germ line KIT mutations. The available data are consistent with a role for KIT mutations as the initiating oncogenic events in most sporadic GISTs, but this is unproven. In contrast, the characteristic cytogenetic aberrations in GISTs, which include deletions of chromosomes 14 and 22, appear to be secondary alterations. Among the chromosomal alterations are several found in most malignant GISTs, but rarely in low-grade GISTs. Thus cytogenetic markers might play a future role in the diagnostic and prognostic evaluation of GISTs. Although the cytogenetic profile in GISTs is often distinctive, featuring a relatively noncomplex karyotype with characteristic chromosomal deletions, none of the individual chromosomal aberrations is specific for GIST. For this reason, cytogenetic studies are less crucial than histopathology, KIT immunohistochemistry, and KIT molecular analyses in the routine evaluation of GISTs.

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