Review Article

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Gastrointestinal Stromal Tumors (GISTs): Definition, Occurrence, Pathology, Differential Diagnosis and Molecular Genetics*

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Gastrointestinal stromal tumor (GIST) is now defined as a specific, KIT-expressing and KIT-signaling driven mesenchymal tumor of the gastrointestinal (GI) tract. The specific identification of GIST has become more important after the availability of KIT-selective tyrosine kinase inhibitor Imatinib mesylate, STI571, commercially known as Gleevec/Glivec™ (Novartis Pharma, Basel, Switzerland) in the treatment of unresectable and metastatic tumors. GISTs are the most common mesenchymal neoplasms of the GI tract, and encompass most tumors previously classified as gastric and intestinal smooth muscle tumors. GISTs typically present in adults over 40 years (median age 55 - 60 years) and only exceptionally in children . They can present anywhere in the GI-tract from the lower esophagus to the anus. A great majority of GISTs occur in the stomach (60 - 70%) or small intestine (25 - 35%). Colon, rectum, appendix (together 5%) and esophagus (2 - 3%) are rare sites. Some GISTs are primary in the omentum, mesentery or retroperitoneum, unrelated to the tubular GI-tract, but most GISTs in these sites are metastases from gastric or intestinal primary. Histologically GISTs vary from cellular spindle cell tumors to epithelioid and pleomorphic ones, and morphology differs somewhat by site. By definition, GISTs are KIT(CD117)-positive. Positivity for nestin (90 - 100%) and CD34 (70%) are also characteristic but less specific features. Smooth muscle actins (20 - 30%) and heavy caldesmon (80%) are often expressed, whereas desmin is usually absent. Predictive of malignancy are mitotic rate over 5 per 50 HPF or size over 5cm. However, mitotically inactive intestinal tumors can metastasize, and gastric tumors are in average less often malignant than the intestinal ones. True smooth muscle tumors, GI-schwannoma and undifferentiated sarcomas are the most important differential diagnoses. KIT activating mutations occur in 70 - 80% of cases. Their signaling consequences, clinical correlation and response to tyrosine kinase inhibitors, and specific genetic alterations are under intense investigation. Majority of these mutations are in-frame-deletions and missense mutations clustering in the 5'-end of juxtamembrane domain (exon 11). A rare mutation, an Ala^{502}-Tyr^{503} duplication in exon 9, is specific for intestinal GISTs.

Introduction

Gastrointestinal stromal tumor (GIST) is the term for a specific, immunohistochemically KIT-positive mesenchymal neoplasm of the gastrointestinal (GI) tract and abdomen. GISTs constitute a majority of GI mesenchymal tumors. Pathologic activation of KIT signal transduction appears to be a central event in GIST pathogenesis [40, 42, 88].

The identification and proper definition of GIST has become more important after introduction of targeted treatment with KIT tyrosine kinase inhibitor Imatinib mesylate, STI571, commercially known as Gleevec/Glivec™ (Novartis Pharma, Basel, Switzerland) for metastatic and unresectable GISTs. The early results on isolated cases and clinical trials have shown tumor stabilization or regression in a great majority of metastatic and unresectable GISTs expanding the trials to large numbers of patients with malignant GISTs [25, 48, 99]. The purpose of this review is to clarify the definition, clinical and histopathologic spectrum, molecular pathology and differential diagnosis of GIST.

Definition and Histogenesis of GIST

GISTs are KIT-positive spindle cell, epithelioid, or rarely pleomorphic mesenchymal tumors with characteristic histologic features and occurring anywhere in the GI tract or abdomen. By this definition, a large majority of GI-mesenchymal tumors are GISTs.

Many but not all GISTs have KIT activating mutations. These mutations are believed to cause constitutional activation (phosphorylation of certain tyrosine residues) of the KIT tyrosine kinase independent of the ligand (stem cell factor) binding; the latter is the normal mechanism of KIT activation. KIT activation, in turn, leads to activation of the KIT signaling pathway which ultimately leads to activation of cellular proliferation. In regard to definition, demonstration of a KIT mutation supports the diagnosis of GIST, whereas lack of mutation does not exclude it.
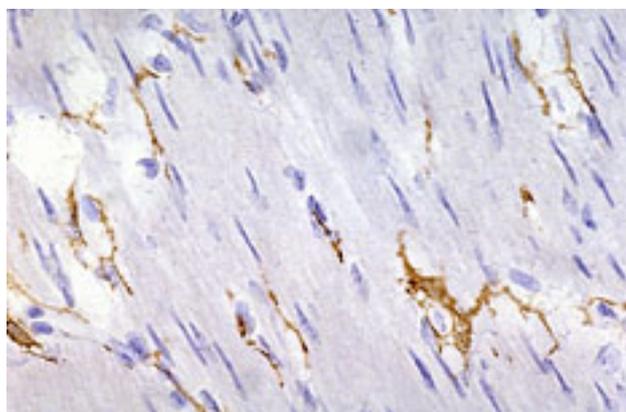

Fig. 1. Cajal cells are identified as slender spindle cells with thin, elongated processes, seen here in the muscularis propria of colon.

GIST share a phenotypic similarity with the KIT-positive Cajal cells (interstitial cells of Cajal) of the GI tract located around the myenteric plexus and dispersed in the muscularis propria. These cells were identified as KIT-dependent and KIT-positive cells which act as intermediaries between the GI nervous and smooth muscle systems. Their functions include generation of pacemaker activity and inhibitory neurotransmission [71, 106, 112]. They are identified as KIT-positive slender cells with thin, elongated processes around the myenteric plexus or between smooth muscle fibers (Fig. 1).

Two observations strongly suggest that Cajal cells or subsets thereof represent multipotential stem cell-like population, which is the logical candidate for histogenesis of GIST. First, Cajal cells have the capability to differentiate into smooth muscle, if deprived of the KIT-signaling [122]. Secondly, experiments on the development of chimeric birds and mice have shown that the Cajal cells and smooth muscle cells originate from a common precursors [59, 134].

Relationship of GIST with previous terminology

GISTs comprise a great majority of tumors formerly diagnosed as leiomyomas, leiomyosarcomas, leiomyoblastomas and smooth muscle tumors of the GI-tract and adjacent abdominal sites [88]. Esophageal and colorectal muscularis mucosae leiomyomas are the two most important exceptions (Table 1). Gastrointestinal autonomic nerve tumors, GANTs [57] are now understood as ultrastructural variants of GIST [60], based on their histologic and immunohistochemical similarity with GISTs, KIT-positivity and occurrence of GIST-specific KIT-mutations. Because a large majority of mesenchymal tumors of the GI-tract are GISTs, older data on GI leiomyomas and leiomyosarcomas largely reflect GIST data.

Occurrence of GIST

By far, GIST has emerged as the most common mesenchymal tumor of the GI-tract and intra-abdominal soft tissues where GISTs most commonly metastasize. Although the age and sex distribution and sites of occurrence are well-studied in numerous clinicopathologic series, there is less exact data on the population incidence.

Incidence

Based on a population sample from Southern Finland, we have estimated the incidence of malignant GISTs as 4 per million and total incidence perhaps 10 times as high [88]. Similarly, a recent study from southwestern Sweden has determined the population incidence of clinically manifest GIST as 20 per million [52]. Based on these studies, the total incidence of GISTs is 20 - 40 per million. The prevalence of GISTs is much higher, since many malignant tumors have a long clinical course of 10 - 15 years of duration.

Age and sex

According to all larger clinicopathologic series, GISTs have a predilection to adults over 50 years of age, with the median ages varying between 55 - 65 years in different sites with no clear sex predilection. The proportion of patients under 40 years have ranged between 5% and 20% in the larger clinicopathologic series. GISTs are extremely rare in children, and we have seen only isolated cases.

The majority of pediatric GISTs reported in the literature have been diagnosed in the second decade in the stomach [63]. However, tumors classified as GISTs in infants more likely represent inflammatory myofibroblastic tumors; this seems to be the case with a recently reported KIT-negative tumor [7].

Site of occurrence

GISTs occur throughout the tubular GI-tract from the lower esophagus to the anus. The most common site is by far stomach (60 - 70%) followed by small intestine, rectum and colon. Only small numbers of cases have been reported in the esophagus and appendix [77, 85]. The estimated frequency of GISTs at different sites is shown in Table 2.

A number of primary GISTs have been reported outside the GI-tract proper in the abdomen, specifically in the omentum, mesenteries, and retroperitoneum [75, 104]. However, more often GISTs in these sites are metastatic from the GI-tract. A number of GISTs are diagnosed as disseminated intra-abdominal tumors involving multiple intestines, peritoneal surfaces and other abdominal organs. In such cases, the primary site is often impossible to determine.

Hematogenous metastases commonly develop in the liver, and rarely in bones and lungs [24, 96]. Rarely, we have seen GISTs as metastases in peripheral soft tissues, such as arm, axilla and abdominal wall. Such metastases can be clinically confusing, especially when diagnosed disconnected from the history of a previous GIST.

TABLE 1

Extrapolation of the previously employed diagnostic terminology to the present terminology related to GISTs

Old terminology	Present terminology and comment
Esophageal leiomyoma	Most of these tumors are true leiomyomas, histologically and clinically separate from GISTs
Esophageal leiomyosarcoma	Most of these tumors are GISTs, and a small minority are true leiomyosarcomas
Gastric leiomyoma	Great majority are GISTs, and very few are leiomyomas similar to those more commonly seen in the esophagus
Gastric leiomyoblastoma	Corresponds to epithelioid GIST
Gastric leiomyosarcoma	Great majority are GISTs
Small intestinal leiomyoma and leiomyosarcoma	Great majority are GISTs
Colonic and rectal leiomyoma	The small tumors involving muscularis mucosae only are true leiomyomas (benign) Some tumors externally involving colon and rectum in women are uterine type leiomyomas with estrogen and progesterone receptor positivity Most intramural tumors are GISTs, and very few are true leiomyomas
Colonic and rectal leiomyosarcoma	Great majority are GISTs. Small minority are true leiomyosarcomas (see Table 2)
Gastrointestinal autonomic nerve tumor (GANT)	This category merges with GIST, representing its ultrastructural variant
Leiomyosarcoma of omentum and mesentery	A majority of these tumors are GISTs and a minority are true leiomyosarcomas
Retroperitoneal leiomyosarcoma	Includes up to one third of GISTs, primary from stomach or intestines, and rarely primary in the retroperitoneum. Clinical and gross pathology correlation is needed to determine the primary site

GIST as a Part of Tumor Syndromes

The three tumor syndromes with GIST as a manifestation are familial GISTs, Carney's triad and neurofibromatosis 1 (NF1). The patients with familial GISTs have inheritable germline mutations in KIT, but molecular pathogenesis of GIST in the two latter syndromes is unknown. Only a small minority of GIST patients have one of these syndromes.

Familial GISTs

Six families with KIT positive multiple GISTs have been reported and as expected, the pattern of inheritance was autosomal dominant. Constitutive KIT mutations have been documented in the members of these families [8, 43, 45, 47, 72, 97] representing 5 different types of KIT mutations affecting juxtamembrane or I and II tyrosine kinase domains (Table 3). Patients with familial GISTs are usually diagnosed with multiple GISTs occurring at younger age than sporadic GISTs. Some patients had other manifestations of KIT activation in mast cells (urticaria pigmentosa), melanocytes (cutaneous hyperpigmentation) or both [8, 72, 97].

Neurofibromatosis 1 and GIST

Neurofibromatosis 1 is one of the most common autosomal dominant disorders with a birth incidence of approximately 1:3000, but approximately half of the cases results from new mutations. This syndrome leads to formation of multiple neurofibromas, and also entails spinal and craniofacial skeletal abnormalities. There is a definite although relatively small (1 - 4%) risk of malignant peripheral nerve sheath tumor arising in neurofibroma and risk of central nervous system tumors, especially gliomas [103].

GISTs have been sporadically diagnosed in patients with NF1 [116, 128]. Based on larger studies, they occur in this syndrome by a non-random association, are often multiple and usually located in the small intestine [51]. In our series of 156 duodenal GISTs, ten patients (6.4%) had NF1 syndrome. Most of these patients had multiple small intestinal GISTs. Some tumors had cytologic atypia, but clinicopathologically the group was heterogeneous including patients with long-term survival (despite multiple GISTs) and those who died of metastatic tumor. Severe GI hemorrhage resulting in patient death can be a complication in NF1 patients with multiple ulcerated GISTs [89].

Carney's triad and related syndromes

This syndrome, originally described by Carney from the Mayo Clinic, includes gastric GIST, paraganglioma and pulmonary chondroma and adrenal cortical adenoma (with at least two of these tumors in the patient by definition). Based on a review on 79 such patients by Carney [12], all GISTs were gastric. The tumors often occurred at a young age (mean 20 years), and there was a striking female predominance (85%) and a majority of tumors had an indolent behavior. However, 41% of the patients experienced a local

TABLE 2

Relative frequencies of the occurrence of GISTs at different sites, and estimated frequencies of malignant tumors at different sites

Site	Estimated percentage of all GISTs	Estimated frequency of malignant behavior
Esophagus	1-3%	Great majority
Stomach	60-70%	25%
Small intestine	25-30%	50%
Duodenum	5%	30-40%
Colon	1%	Great majority
Rectum	5%	30-40%
Omentum and mesenteries	<5%	50%
Widely metastatic in abdomen	5%	–

TABLE 3

KIT mutiation in familial GISTs and clinical symptoms related to pathological KIT activation reported in GIST families

KIT mutation	Dysphagia	Cutaneous hyperpigmentation	Mast cell tumors	Reference
Substitution of Arg for Trp[557]	NO	NO	NO	43
Substitution of Ala for Val[559]	NO	YES	NO	72
Deletion Val[559] or Val[560]	NO	YES	NO	97
Substitution of Ala for Val[559]	NO	YES	YES	8
Substitution of Lys for Gly[642]	NO	NO	NO	47
Substitution of Tyr for Asp[822]	YES	NO	NO	45

recurrence, and many had liver metastases, but nevertheless often survived long, even with metastatic disease. There was only 13% of disease-related mortality.

We have seen only a handful of such cases, including a young woman with pulmonary chondroma and epithelioid GISTs diagnosed simultaneously, and a 26-year old man who had an epithelioid gastric GIST with a local gastric recurrence five years later and two paragangliomas (carotid body and vagal tumors) 25 years from the first surgery. Our experience indicates that these GISTs are KIT-positive tumors with epithelioid morphology.

Recently, Carney et al. [13] suggested that there is another syndrome separate from Carney's triad that includes gastric GIST and paraganglioma, with more emphasis on the latter. These GISTs occurred at a young age and were considered malignant based on omental seeding. However, the course was rather indolent with some patients experiencing recurrences over the span of more than 40 years [13].

Clinical Presentation of GIST

Small GISTs are typically incidentally detected on the external aspect of stomach or intestines during radiologic studies or surgery for unrelated conditions. Small rectal GISTs are often palpated as intramural nodules during a routine prostate or gynecologic examination. Less frequently, GIST is an incidental endoscopic finding.

Gastrointestinal bleeding or vague ulcer-like pain are the most common symptoms of GIST. Many of these patients have anemia due to chronic bleeding, and in some patients localization of GIST follows extensive clinical and radiologic studies. Those GISTs that do not cause ulceration can grow into a large size with little symptoms. Some of these tumors form palpable abdominal masses, and occa-

sionally, GIST causes an intestinal perforation. Sometimes GIST is detected as a disseminated intra-abdominal tumor.

Pathology of GISTs - Gross Pathology

Preoperative radiologic studies by CT scan or magnetic resonance imaging are very helpful in determining the tumor configuration and its extension and relationship with adjacent organs. In general, externally bulging tumors are more common than intraluminal masses, and only some small GISTs are detected as purely intramural tumors, or rarely, as intraluminal polyps. Different gross patterns can be observed. They include hemispherical submucosal or serosal nodules, large cystic tumors, pseudodiverticles, and rarely, plaque-like masses [62].

Small to medium-sized gastric GIST typically form a well-delineated spherical or hemispherical mass beneath mucosa pushed into the lumen to form a smooth-contoured elevation. Focal mucosal ulceration is common in GISTs of all sites, and is not related to tumor malignancy. In the intestines, any larger GIST typically forms an outward bulging mass. Some small intestinal examples form asymmetric dumbbell-shaped masses with a smaller intraluminal and a larger externally bulging components. Some gastric and intestinal GISTs form a spherical or hemispherical serosal nodule, attached to the wall with a variably broad base or only by a narrow pedicle.

Large GISTs in the stomach and intestines often form an externally bulging masses, whose extensive extra-GI component can mask the tumor origin from the stomach or intestines. These tumors are often centrally necrotic and cystic containing hemorrhagic-necrotic material or fluid and viable tumor only as a narrow peripheral rim. Some intestinal GISTs form pseudodiverticles as a result of fistulation of a

necrotic tumor into the intestinal lumen. Such tumors arising in the ileum have been sometimes thought to represent GISTs arising from Meckel's diverticle, but we have not yet seen a histologically convincing origin form this vestigial structure.

Duodenal GISTs involving the periampullary region can extend to the pancreatic head region immediately adjacent to external wall of duodenum and clinically and radiologically simulate a primary pancreatic tumor.

Small GISTs of the colon rectum can bulge inward forming an intraluminal polyp. Larger rectal GIST often grow into the rectovaginal septum in women and are attached to the prostate in men, sometimes clinically simulating a prostatic tumor; cyst formation is seen in some of such larger tumors. However, close examination of these cases reveals involvement of the rectal muscular layer.

Extra-GI tract location in the omentum, mesenteries, retroperitoneum or urinary bladder serosa is possible [75, 104]. More commonly, GISTs in these locations represents intra-abdominal metastases from gastric or intestinal primaries. Usually these nodules are spherical with smooth surfaces, and in rare instances, innumerable pea-sized peritoneal nodules are present. Search of primary origin from stomach or intestines is always necessary for the apparently extra-GI GISTs.

Small GISTs are often firm and sometimes rubbery, whereas larger and malignant GISTs tend to be soft with fish-flesh or lymphoma-like consistency. Gross calcification is rare, usually seen in small tumors. On sectioning, the viable tumor areas are most commonly pink-tan, and less often gray, off-white or pale yellowish. GISTs are often hemorrhagic-appearing with a porous or microcystic texture, and the contents of larger cysts vary from fluid to loose hemorrhagic-necrotic material.

Histologic Features of GISTs

The histologic features of GIST vary, and to some degree this variation is site-dependent. Most commonly, GISTs have a spindle cell pattern (60 - 70%), whereas epithelioid cytology is seen in 20 - 30% of cases exclusively or focally, and a pleomorphic pattern rarely (<5%). In all GI-sites, GISTs often grow between bundles of smooth muscle fibers often creating a micronodular, plexiform pattern. Examples of the most common histologic patterns of KIT-positive GIST are shown in figure 2.

Cytologically, the cell borders vary from distinct to syncytial pattern; the former is common in epithelioid tumors, and the latter often seen in spindle cell GISTs. The nuclei typically have an evenly dispersed chromatin, but some tumors contain prominent nucleoli in varying numbers of cells. The nuclei are often elongated with more pointed ends, but some GISTs show blunt-ended, cigar-shaped nuclei similar to those typically observed in leiomyosarcomas.

The mitotic counts in GISTs vary in numbers from 0 - 20 per 50 HPF, and high mitotic counts are rare; the mitotic count is one of the most important histologic predictors of malignancy. The mitotic figures are usually regular, and markedly atypical forms are quite rare.

Histologic patterns of gastric GISTs

The majority of gastric GISTs are highly cellular spindle cell tumors, often having a more basophilic appearance than leiomyomas because of the high nuclear density and scant cytoplasm. Some small GISTs have tumor cells dispersed in a prominent, amorphous collagenous background which sometimes contains extensive hyalinization (Fig. 2A), sometimes with stromal calcifications. Perinuclear vacuolization is a common feature of gastric GISTs, and sometimes it is prominent throughout the tumor (Fig. 2B).

Prominent nuclear palisading is a common feature often seen in gastric and sometimes in colorectal GISTs but less commonly in small intestinal tumors. It can be seen focally or throughout the tumor, and such palisades can form long fronts. The palisades often resemble the Verocay bodies of peripheral schwannomas and they can occur in both benign (Fig. 2C) and malignant GISTs (Fig. 2D).

In some gastric GISTs the tumor cells are seen as bundles or narrow fascicles separated by fibromyxoid or hyalinized stroma, sometimes even resembling paraganglioma-like compartmental pattern (Fig. 2E). Some malignant GISTs with extensive microscopic necrosis have the remaining viable areas grouped as perivascular collars resembling those often seen in malignant peripheral nerve sheath tumors. A pattern of intersecting fascicles resembling that often seen in leiomyosarcoma, is common (Fig. 2F).

The epithelioid GISTs of the stomach correspond to the previous designation of leiomyoblastoma. They are typically composed of polygonal cells with round nuclei and ample cytoplasm varying from eosinophilic to amphophilic and clear, and have a spectrum from benign to malignant (Fig. 2G); however the former are more common. Focal nuclear pleomorphism is relatively common in these tumors, but a small minority of gastric GISTs (<3 - 5%) have extensive nuclear pleomorphism (Fig. 2H). However, most tumors with marked pleomorphism are not GISTs, but rather represent pleomorphic leiomyosarcomas or undifferentiated sarcomas, and as such can be classified as malignant fibrous histiocytomas.

Histologic patterns of intestinal GISTs

Most small intestinal GISTs have a spindle cell pattern. Approximately half of the GISTs from duodenum to the ileum contain microscopically distinctive, round, oval or elongated eosinophilic and PAS-positive aggregates of extracellular collagen fibers (Fig. 3A). These have a lamellar, concentric, skein-like ultrastructural appearance and hence
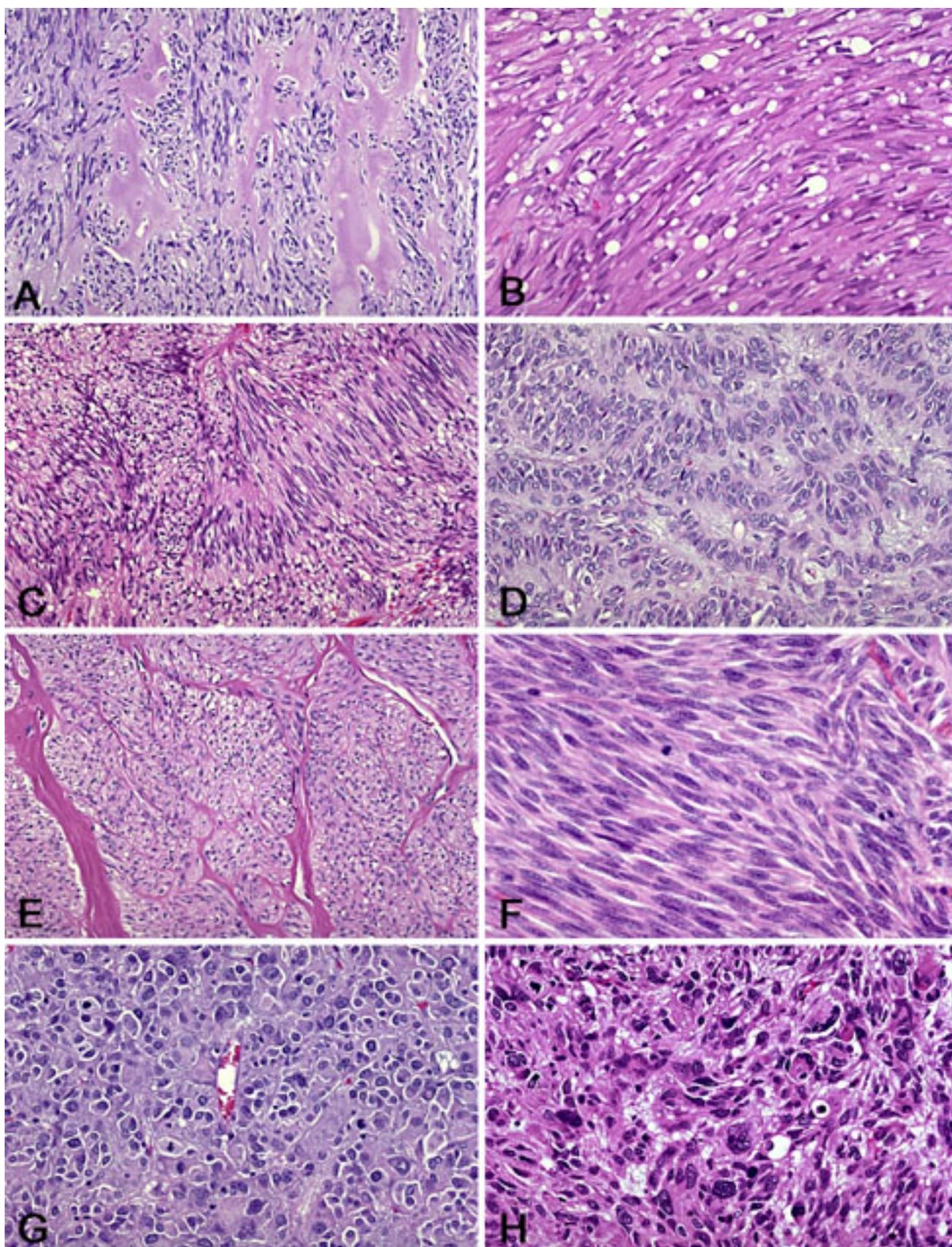
Fig. 2. Histologic spectrum of gastric GISTs. A. Benign gastric GIST with collagenous background, only moderate cellularity and no mitotic activity. B. Prominent perinuclear vacuolization is common in gastric GISTs. C. Benign but cellular gastric GIST with nuclear palisading reminiscent of a schwannoma. D. A malignant gastric GIST with nuclear palisading. E. A compartmental pattern is relatively common in gastric and intestinal GISTs. F. Malignant GISTs can have a fascicular pattern remiscent of that in leiomyosarcoma. G. Epithelioid gastric GISTs have a spectrum from benign to malignant; this tumor was malignant. H. Pleomorphic histology is a rare finding in GISTs.

have been named as skeinoid fibers [90]. These structures are especially seen in the non-malignant examples, and have been found as a statistically favorable prognostic feature in some studies [89]. Vascular hyalinization and calcification are relatively common, especially in small, benign tumors (Fig. 3B). Peculiar hemangioma-like vascular proliferation may focally obscure the cellular elements of GIST (Fig. 3C). Focal nuclear pleomorphism is more common in small intestinal GISTs of NF1 patients (Fig. 3D). Trabecular-myxoid pattern is more common in malignant mitotically active GISTs and may also occur in stomach (Fig. 3E). The epithelioid cytology in intestinal GISTs is essentially limited to malignant tumors, and differs both morphologically and clinically from the gastric counterparts (Fig. 3F).

Histologic features of GISTs of other sites

Most GISTs of sites other than stomach and small intestine have a spindle cell pattern. The rarely reported appendiceal GISTs resemble the small intestinal ones by the common content of skeinoid fibers, which are also seen in some colonic GISTs but not in rectal GISTs.

Rectal spindle cell GISTs can show hyalinized-calcified or palisading nuclear pattern somewhat similar to those seen in gastric tumors, and malignant examples can have a leiomyosarcoma-like fascicular pattern. Epithelioid GISTs similar to those often seen in stomach are rarely observed in the rectum. In this location, they can be clinically benign tumors similar to their gastric counterparts.

The GISTs in the omentum can have spindle cell and epithelioid features resembling those of gastric GISTs, whereas mesenteric GISTs often have features resembling the small intestinal tumors, including the presence of skeinoid fibers in some examples.

Immunohistochemical Features of GIST

The key feature of GIST is KIT-positivity [42, 50, 114, 119], and currently no equal surrogate markers have been established. Suggested guidelines for performing KIT immunostaining to comprehensively capture all GISTs are shown in Table 4. KIT-positivity is a major diagnostic feature and also is a clue to presumed histogenesis from the Cajal cells or related stem cells. Other antigens commonly expressed but less specific for GISTs are CD34 and nestin. GISTs are variably positive for smooth muscle markers: smooth muscle actin, heavy caldesmon, calponin, and embryonic smooth muscle myosin, but are generally negative for desmin. Positivity for S100 protein is rare, and GFAP is always absent. Like some other sarcomas, keratins 18 and 8 are variably expressed. The markers useful in the diagnosis and differential diagnosis of GIST are listed in Table 5.

Presently, KIT-positivity supersedes the histogenetic significance of the other markers, and it does not seem to be indicated to separate GISTs into histogenetic subtypes based on expression of actins and S100-protein.

KIT

KIT-positivity in GISTs is typically strong and global. Membrane staining is often present, and this pattern is more readily observed in epithelioid GISTs. Many GISTs also have paranuclear KIT-positive dots ("Golgi-zone pattern"), and spindle cell tumors usually have a pan-cytoplasmic appearing staining pattern, probably because membrane staining in these cells is difficult to observe due to the narrow cross dimension of the spindle cells (Fig. 4A, B). In our experience, some epithelioid GISTs of the stomach are less uniformly and sometimes only weakly positive for KIT; the molecular correlation of this finding is under investigation.

KIT antibodies that show a high specificity should be used. Fibroblasts and normal smooth muscle cells should be KIT-negative. If avidin-biotin based detection system is used, avidin biotin block is necessary to eliminate the detection of endogenous biotin. This is present, for example, in hepatocytes, some other epithelial cells, and importantly, in some tumor cells, causing potential false positive staining. According to our experience, the best ones currently available for formalin-fixed and paraffin embedded tissue are polyclonal antibodies. The monoclonal antibodies available react inconsistently in formalin-fixed and paraffin-embedded tissue and identify only a minority of GISTs.

The normal KIT-positive components, mast cells and Cajal cells, are important positive controls to validate the sensitive detection of KIT including the adequacy of heat-induced epitope retrieval.

CD34

Approximately 70 - 80% of GISTs are positive for CD34, the hematopoietic progenitor cell antigen also expressed in endothelial cells, subsets of fibroblasts and many neoplasms related to these cell types [80]. This marker is closest to a surrogate marker to KIT (Fig. 4C). The frequency of CD34-positivity varies by site. GISTs of esophagus and rectum are nearly consistently CD34-positive (95 - 100%). There is no difference in the frequency of CD34-expression between benign and malignant GISTs, and site-specific studies do not show significant survival differences between positive and negative cases [83, 89]. The difference in CD34-positivity between GISTs of different sites has been explained by hypothetical origin from CD34-negative and positive subsets of Cajal cells [106].

Smooth muscle actin

Older studies predating KIT immunohistochemistry noted that stromal tumors separate from leiomyomas could
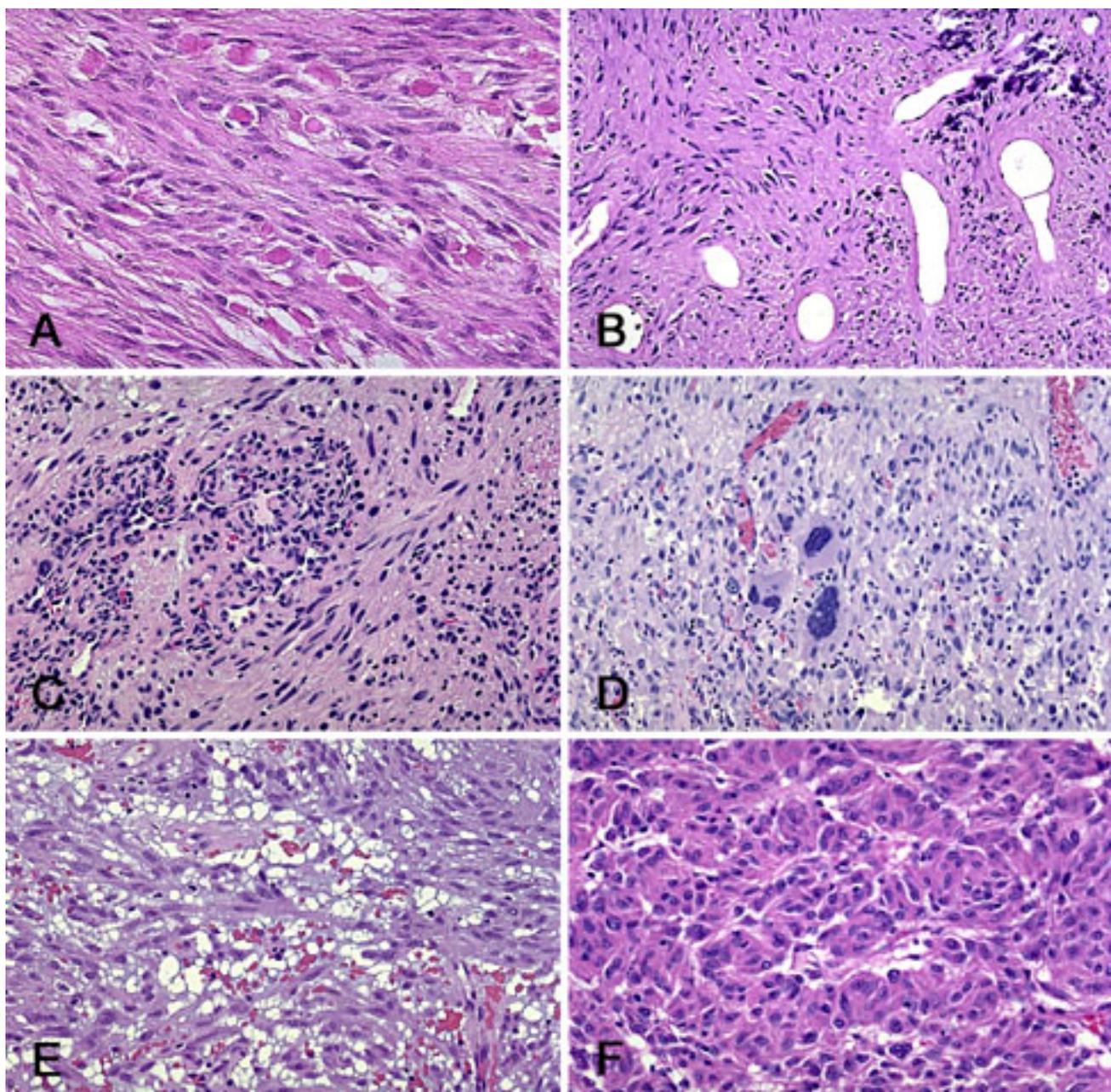
Fig. 3. Histologic spectrum of intestinal GISTs. A. Benign small intestinal GIST with a relatively low cellularity and skeinoid fibers. B. Low cellularity, hyalinized vessel walls and calcifications are seen in some small intestinal GISTs. C. Hemangioma-like vascular proliferation may be prominent focally obliterating the cellular components of small intestinal GISTs. D. Marked, focal nuclear pleomorphism can occur in GISTs of patients with NF1 syndrome. E. A small intestinal GIST with a trabecular and myxoid pattern. F. Malignant epithelioid GIST from colon.

be actin positive [32]. Large series have shown approximately 30% of GISTs, especially gastric and small intestinal tumors, to be positive for smooth muscle actin, whose expression is often reciprocal with that of CD34; sometimes this is seen in one tumor where CD34-positive and actin negative areas and CD34-negative and actin-positive areas are present [80]. The actin-positivity varies from focal to extensive, and can be equally prominent as KIT-positivity in these tumors (Fig. 4D).

Desmin

Positivity for desmin, the muscle type intermediate filament protein, is rare in GISTs of all sites, but has been observed relatively more often among esophageal GISTs [77]. However, only 3% of gastric GISTs and none of the small intestinal GISTs were positive in our survey of nearly 300 GISTs [80]. Desmin-positivity can occur in epithelioid GISTs, usually limited to small numbers of tumor cells.

TABLE 4

Guidelines when to perform KIT immunostaining for comprehensive detection of GIST

1)	Primary mesenchymal tumors of the gastrointestinal tract, with the possible exception of typical smooth muscle tumors, and schwannomas, especially when the investigator is experienced
2)	Spindle cell, epithelioid (and pleomorphic) neoplasms of the omentum, mesentery and retroperitoneum, with the possible exceptions of well-differentiated leiomyomas, and schwannomas, especially when the investigator is experienced
3)	Unclassified tumors of the abdomen and abdominal wall
4)	Mesenchymal tumors of the liver
5)	Peripheral soft tissue tumors suspicious of GIST metastases

TABLE 5

Immunohistochemical differential diagnosis of the most important mesenchymal tumors of the GI-tract. Key: + positive; +/– positive or negative; –/+ negative, a minority of cases positive; –/(+) negative, occasionally positive

	KIT	CD34	Nestin	SMA	Desmin	S100	GFAP
GIST	+	+/-	+	–/+	–/(+)	–/(+)	–
Leiomyoma	–	–	–	+	+	–	–
Leiomyosarcoma	–/(+)	–/(+)	?	+	+/(–)	–	–
Schwannoma	–	–/(+)	+	–	–	+	+
Undifferentiated sarcoma	–	–	?	–/(+)	–	–	–

Other smooth muscle markers

Embryonic form of smooth muscle myosin [110] and heavy caldesmon, an actin-binding cytoskeletal protein [76], are smooth muscle antigens regularly expressed in GISTs (Fig. 4E). The expression of these antigens in GIST suggests is consistent with the origin from Cajal cells that have potential to differentiate into smooth muscle cells if KIT pathway is blocked [122].

Common GI smooth muscle infiltration of GISTs results in numerous entrapped actin- (and desmin-) positive intratumoral spindle cells, which should not be confused with actin- and desmin-positive tumor cells in the immuno-phenotyping (Fig. 4F).

S100 protein

S100 protein expression is relatively rare in GISTs, and occurs more commonly in the small intestinal GISTs (10%). The positivity is usually focal, but is present in both cytoplasm and nuclei, similar to S100 protein expression in many other S100-positive tumors [80].

Nestin

Recently, GISTs were reported consistently positive for nestin, a type VI intermediate filament protein typical of many stem cells, including those of nervous and muscular systems [123]. We have confirmed consistent nestin-expression in nearly all GISTs independent of site, histologic pattern and malignancy, but have also found nestin equally often in GI schwannomas [115]. Nestin is also expressed in other tumors, such as rhabdomyosarcomas and melanomas indicating its incomplete specificity for GIST.

Keratins

Keratin-positivity, in our experience, can be sometimes seen in GISTs with antibodies reacting with keratin 18 and, to lesser degree, to keratin 8, but keratins 7, 13, 14, 17, 19 and 20 are not present. Keratin 18-positivity is more common in malignant GISTs. For example, 27% of gastric GISTs with documented malignant behavior were keratin 18 positive, whereas only 8% of benign tumors were positive [115].

Other markers

Like most mesenchymal tumors, especially the malignant ones, GISTs are positive for vimentin. They are uniformly negative for glial fibrillary protein (GFAP) which helps to separate them from GI schwannomas, tumors that are usually GFAP-positive. Neurofilament 68 occurs in a subset of GISTs (<10%). The biologic significance of this observations is presently unclear [89].

Tumor Behavior and Prognostic Factors

The largest clinicopathologic series indicate that GISTs have a spectrum from small benign, typically incidentally diagnosed nodules to overt sarcomas at all sites of occurrence [5, 83, 88]. The series reported from oncologic hospitals are biased toward malignant tumors, and this may give an incorrect impression of GISTs as generally malignant
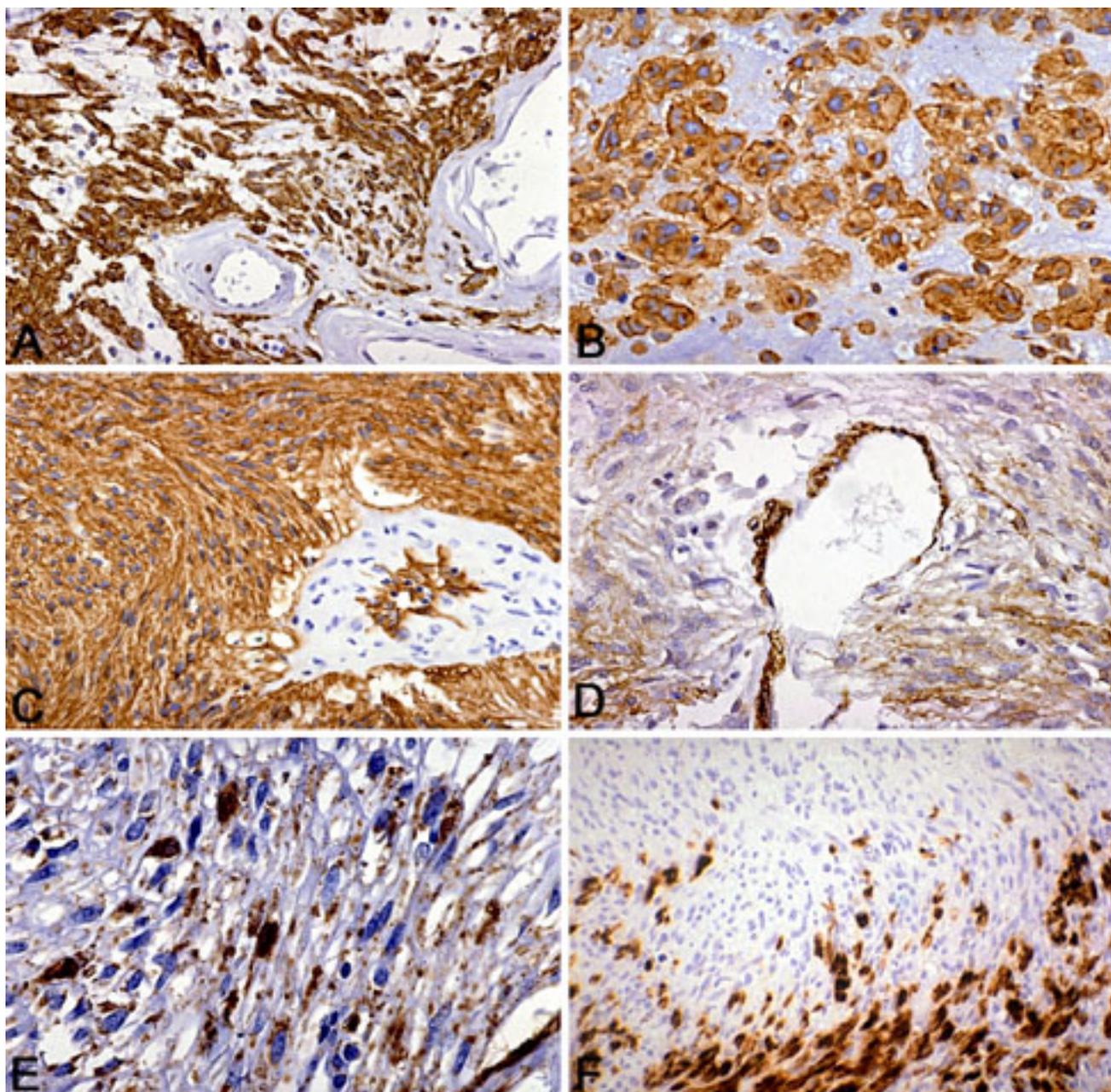
11

Fig. 4. Immunohistochemical spectrum of GIST. A. Apparent diffuse KIT positivity in a spindle cell GIST. B. KIT positivity with a perinuclear dot-like pattern. C. A rectal GIST with uniform CD34-positivity, also seen in vascular endothelia. D. Smooth muscle actin positivity occurs in one third of GISTs. E. More commonly, smooth muscle actin positive cells within the GISTs represent entrapped normal smooth muscle cells, typically seen in a streaming pattern. F. Heavy caldesmon-positivity is seen in a majority of GISTs.

tumors. The relative frequencies of benign and malignant GISTs varies by site (Table 2), and of these differences, the apparently more benign behavior of gastric vs. intestinal GISTs is the most significant. Whether this is based on detection of the gastric tumors at an earlier phase of development, or on intrinsic differences in the behavior of gastric vs. intestinal GISTs, is unclear.

Although predictably benign and malignant groups can be defined by histologic criteria, the prediction of tumor behavior is difficult for some GISTs. The following guidelines have been suggested (Table 6).

Benign GISTs

The combination of size 2cm or less with the low mitotic rate translates to benign clinical course according to follow-up studies of 10 - 20 such tumors in the stomach [80], duodenum [89] and rectum [83]; in the latter site one small tumor recurred probably representing regrowth of primarily incompletely excised tumor [83]. Small benign solitary GISTs should not be confused with a situation where multiple small GIST nodules have disseminated from a malignant GIST.

TABLE 6

Guidelines for the evaluation of malignancy of GISTs. Modified from Miettinen et al. [ref. 87]

Probably benign
Intestinal tumors
Maximum diameter less than 2cm and
no more than 5 mitoses per 50 HPF
Gastric tumors
Maximum diameter less than 5cm and
no more than 5 mitoses per 50 HPF
Malignant
Intestinal tumors
Maximum diameter over 5cm or
more than 5 mitoses per 50 HPF
Gastric tumors
Maximum diameter over 10cm or
more than 5 mitoses per 50 HPF
Uncertain or low malignant potential
Intestinal tumors
Maximum diameter 2 - 5cm and
no more than 5 mitoses per 50 HPF
Gastric tumors
Maximum diameter 5 - 10cm or
no more than 5 mitoses per 50 HPF

In duodenal GISTs, the presence of skeinoid fibers and perivascular hyalinization [89] have been found statistically significant features predicting benign behavior.

GISTs of uncertain malignant potential

This group is represented by relative small to medium-sized intestinal tumors (2 - 5cm) with low mitotic activity. However, gastric GISTs seem to behave less aggressively, and size range 5 - 10cm has been considered more appropriate for gastric tumors of this group [87].

Malignant GISTs

Intestinal GISTs that are either larger than 5cm or have mitotic activity exceeding 5 mitoses per 50 HPF show high potential for intra-abdominal and liver metastases. Because of the overall lower potential of gastric tumors, only those over 10cm are included in this predictive category. Statistically significant histologic features that indicate malignancy also include mucosal invasion [10, 125] and presence of coagulation necrosis.

According to the most widely used sarcoma grading systems, malignant GISTs would mostly be graded as low or intermediate grade tumors based on a generally low mitotic rate. A minority (10 - 20%) of these tumors would have the high grade designation based on the combination of high mitotic rate (over 10 mitoses per 10 HPF) and coagulation necrosis. Considering the new specific treatment, traditional grading may not be as important as for other sarcomas.

Proliferation markers

Ki67 analogues (MIB1, Ki-S5) applicable in formalin-fixed and paraffin embedded tissue may assist in tumor evaluation [29, 39]. Tumors with more than 10% of nuclear positivity for Ki-S5 has have been shown to develop metastases and have tumor-related mortality with statistical significance [109]. However, in series of small intestinal GISTs, MIB1-scores were not found helpful in prediction [125].

Differential Diagnosis of GIST

There are two distinct aspects to this problem: the KIT-positive tumors that are not GISTs, and tumors that by their location and overall clinicopathologic features can simulate a GIST. Notably, opinions on KIT-negativity and positivity of some tumors vary; for example, desmoid tumors have been reported as KIT-positive by some antibodies.

Other KIT-positive tumor that are not GISTs

Although a definitional feature, KIT positivity as such is not sufficient for the diagnosis. Consistently KIT-positive other tumors are mastocytoma, seminoma, pulmonary small cell carcinoma, blastic extramedullary myeloid tumor (granulocytic sarcoma), the tissue manifestation of acute myeloid leukemia [16, 124]. However, by their overall clinicopathologic features, these tumors can hardly be confused with GISTs.

Other abdominal or GI tumors that are variably KIT-positive include metastatic melanoma and the related clear cell sarcoma (30 - 50%), Ewing sarcoma family of tumors (50%), childhood neuroblastoma (50 - 80%), angiosarcoma (50%) and some other carcinomas [78, 80, 91]; we have also noted KIT-positivity in some variants of paraganglioma, and especially in the duodenal gangliocytic paraganglioma. According to our experience, these tumors only rarely enter in the differential diagnosis of GISTs. It remains to be seen whether some of these tumors could become targets for KIT inhibitor treatment in the future. Of the non-hematopoietic tumors, the strongest arguments for dependence on KIT signaling has been made for small cell carcinoma.

Tumors that have been reported as KIT-positive without general agreement include desmoid [132] and some other fibroblastic lesions. The positive results described earlier may have been caused by impure antibody preparations, since new affinity purified polyclonal antibodies, in our experience, lack reactivity in desmoid cells and other fibroblasts and myofibrofblasts [81].

Other abdominal mesenchymal tumors that have to be separated from GISTs

Clinicopathologic features of selected intra-abdominal tumors that can simulate GIST are summarized in Table 7.

TABLE 7
Usually KIT (CD117)-negative gastrointestinal tumors that may resemble GISTs clinically or pathologically (all types immunohistochemically tested by us). References are shown in parentheses
*Denotes tumor entities that can be or have been reported as KIT-positive

Intramural leiomyoma (77, 127)	Most common in the esophagus, rare in gastric cardia, and very rare elsewhere in the stomach and intestines. Most examples are relatively small 1 - 4cm, but few reach a large size over 10cm. More often occurs in young patients than GIST. Esophageal tumors have a strong male predominance. Composed of well-differentiated smooth muscle cells, and usually much less cellular than GIST; focal atypia may occur. Smooth muscle actin and desmin-positive and CD117 and CD34-negative
Leiomyoma of muscularis mucosae (82)	Usually endoscopically diagnosed as an incidental diminutive polyp in colon or rectum of older adults. Size averages 4mm and ranges from 1 - 20mm, rarely over 1cm. Composed of well-differentiated smooth muscle cells merging with muscularis mucosae and usually covered by intact mucosa. Focal atypia may occur, but behavior is benign. Immunohistochemically identical with intramural leiomyoma with mature smooth muscle phenotype
Retroperitoneal and peri-intestinal leiomyoma (100)	Occurs nearly exclusively in adult women, histologically similar to uterine leiomyoma. Can form a large retroperitoneal tumor or smaller nodule attached to external aspect of intestines, usually colon or rectum. Positive for actins, desmin and estrogen and progesterone receptors
Leiomyosarcoma* (77, 79, 83, 89)	Rare in stomach and intestines (at most 5 - 10% of GISTs), but retroperitoneum is a common site. Usually occurs in older adults, with a significant female predominance in retroperitoneal tumors which often arise from major vessels, such as vena cava. Intestinal examples can form a polypoid intraluminal mass, others are transmural. Histologically usually composed of well-differentiated smooth muscle cells, but may be focally pleomorphic. Immunohistochemically typically positive for smooth muscle actins and desmin. A minority are positive for CD34; single KIT positive neoplastic cells may occur
Glomus tumor (86)	Conceptually identical with glomus tumor of peripheral soft tissue. Occurs almost exclusively in the stomach in the GI-tract, mostly in the antrum. Round tumor cells arranged around prominent, often dilated vessels. Immunohistochemically positive for smooth muscle actin and negative for desmin. Variably CD34-positive, but is KIT-negative, often with number of positive mast cells
Inflammatory fibroid polyp (38, 73, 105)	Spindle cell lesion, most commonly seen in the small intestine of adults as an ulcerated intraluminal polyp; less common in the stomach and colorectum. Can cause an intussusception of small intestine. Composed of oval or slender spindle cells in highly vascular granulation tissue-like stroma admixed with lymphoid cells and eosinophilic granulocytes. These lesions have a greater cellular heterogeneity than GISTs. Some examples are CD34-positive, but all are KIT-negative. Smooth muscle actin positivity is possible; negative for desmin
Inflammatory myofibroblastic tumor (17, 74)	Occurs especially in children and young adults, may form a gastric or intestinal mass simulating a GIST. More often omental or mesenteric. Many tumors reported as GISTs in children in literature are IMTs. Spindled or slightly epithelioid cells with amphophilic cytoplasm and cytoplasmic processes. Has ALK-gene expression and rearrangements. Also has been referred to as inflammatory fibrosarcoma and earlier as inflammatory pseudoumor
Mesenteric desmoid* (132)	Can be extraintestinal or have GIST-like gastric or intestinal wall involvement. Grossly very firm and white. Histologically composed of fibroblasts and myofibroblasts in collagenous, often focally myxoid background. CD34-negative; can be focally smooth muscle actin and desmin positive
Solitary fibrous tumor (80)	May present on the peritoneal surfaces, pelvis or occasionally in the liver. Collagenous spindle cell tumor, often with a focal hemangiopericytoma-like pattern. Both benign and malignant variants occur. Nearly always CD34-positive and negative for smooth muscle actin and desmin
Gastrointestinal schwannoma (20, 84, 113)	Usually a relatively small (<5 cm), yellow circumscribed submucosal tumor, most commonly in the stomach and secondly in the colon. Slender, often bundled S100-protein positive spindle cells, often in a microtrabecular pattern in an S100-protein-negative fibrous background. Epithelioid variant occurs as polypoid lesions in the colon. GFAP-positivity is typical; this is almost never seen in GISTs
Undifferentiated sarcomas	Malignant gastrointestinal tumors, which do not express any specific cell-type markers and cannot be currently further defined. May grossly simulate GISTs, but histologically often show greater nuclear pleomorphism
Dedifferentiated liposarcoma	Mesenteric, retroperitoneal tumors that may involve intestinal walls in a GIST-like manner. May have myxoid or pleomorphic MFH- or fibrosarcoma-like features. Diagnosis is difficult if fat is not present in the sampled tissue
Metastatic melanoma*	May form a grossly GIST-like tumor with involvement of the layers of the intestines or stomach. Can also be KIT-positive. More often than GIST forms a polypoid intraluminal lesion. Positivity for S100 protein and melanocytic markers (tyrosinase, melanA, HMB45, in various combinations), is diagnostic

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Awareness of the groups of non-GIST abdominal tumors is useful toward the definition and practical diagnosis of GIST. These tumors are almost uniformly negative for KIT, which however, has been reported in some entities sporadically or sometimes seemingly regularly, but is believed to be false positivity. Occasionally, leiomyosarcomas and liposarcomas contain isolated KIT-positive cells [80]. Considering that GISTs are typically globally KIT-positive, the sporadic KIT-positive cells in other tumors are not likely to cause diagnostic confusion.

The definition of GIST excludes true smooth muscle tumors, which are histologically distinctive for their resemblance to smooth muscle and are smooth muscle actin- and desmin-positive. These tumors include intramural leiomyomas, which are most common in the esophagus and are very rare in the stomach and intestines [77, 89, 127]. Leiomyomas of muscularis mucosae usually occur in the colon and rectum as incidentally diagnosed diminutive polyps [82]. Retroperitoneal leiomyomas occur in women. They can be large, but are estrogen and progesterone receptor-positive, mitotically inactive and represent extrauterine equivalents of uterine leiomyomas [100]. Glomus tumors are rare in the GI tract, are identical with their peripheral counterparts, and almost exlusively occur in the stomach [86]. Leiomyosarcomas [77, 79, 83, 89] are rare in the GI-tract, and sometimes occur as intraluminal polypoid masses (Fig. 5A).

Gastrointestinal schwannomas [20, 84, 113] usually occur in the stomach (60 - 70%) or colon (20 - 30%) in older adults, and they are rare in other locations. Nearly all gastric and most intestinal schwannomas are relatively small (<5cm) intramural spindle cell tumors focally or more extensively surrounded by a lymphoid cuff and composed of bundles of spindle cells with focal atypia, intermingled with fibrovascular septa thus differing from peripheral schwannomas (Fig. 5B).

A distinctive subgroup of GI schwannomas is the epithelioid variant seemingly exclusively occurring in the colonic mucosa and submucosa as small intraluminal polyps. These tumors have an added significance as they must not be confused with colonic carcinoma on biopsy [84].

Inflammatory fibroid polyp [73] in our experience is a heterogeneous group of lesions typically forming an intraluminal polyp. These polyps most commonly occur in small intestine of adult patients and can cause an intussusception. The polyps are often ulcerated and highly vascular with a loose somewhat granulation tissue-like texture, and contain numerous eosinophilic granulocytes in the stroma (Fig. 5C). Some lesions of this group, especially those in the stomach, contain perivascular slender spindle cells that are CD34 positive [38, 105].

Inflammatory myofibroblastic tumor whose malignant appearing variants have also been called inflammatory fibrosarcoma, typically occurs in children from infancy on, and in young adults [17, 74]. The prototypic form of this tumor occurs in or around the GI-tract in the abdomen and as such, it can clinically and grossly simulate a GIST, especially when forming an intramural mass in the stomach or intestines. Histologically these tumors have a heterogeneous cellular composition containing large spindle cells with abundant amphophilic or basophilic cytoplasm and conspicuous diffuse lymphoplasmacytic infiltration (Fig. 5D). The tumor cells are negative for KIT and CD34, but can be actin positive. They are often positive for anaplastic lymphoma kinase (ALK); rearrangements of the ALK gene in chromosome 2p23 leading to activation of this kinase is believed to be a central pathogenetic event and diagnostic marker for this tumor [58].

Desmoid tumor is usually easily distinguished from GIST histologically as a collagenous neoplasm with a prominent vascular pattern (Fig. 5E), although grossly it can form a GI tumor with GIST-like intramural involvement. However, on sectioning, desmoids are firm, white tumors [132].
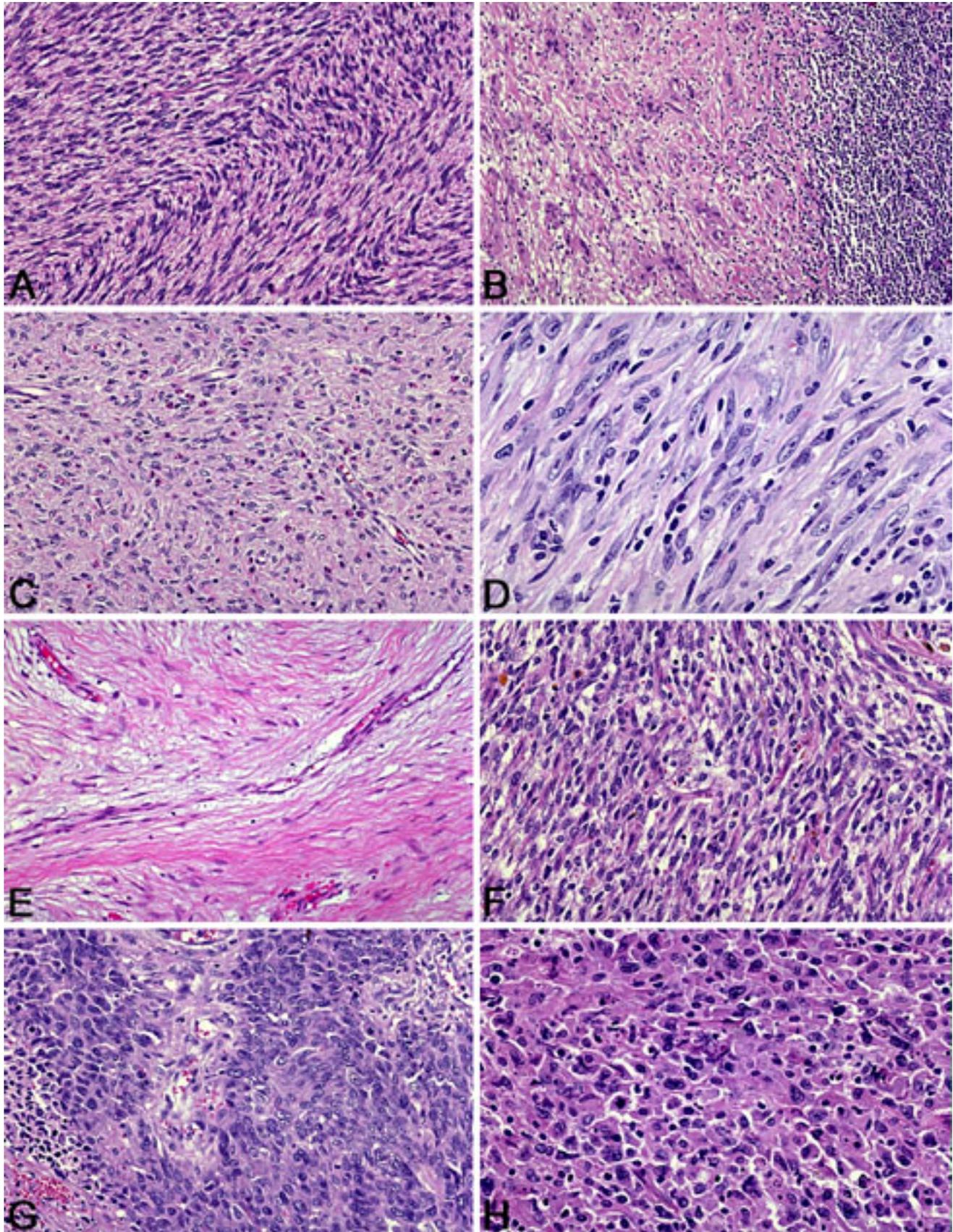
Dedifferentiated liposarcoma in an intra-abdominal location, especially when involving the intestines, can grossly simulate a GIST. However, when involving intestines, these tumors usually form an external mass. Their diagnosis is primarily aided by spindle cell or pleomorphic pattern and proof of previous or simultaneous presence of a lipomatous tumor component.

Undifferentiated sarcoma is a diagnosis by exclusion. These tumors usually have a spindle cell pattern, are more often pleomorphic than GISTs, and may have focal myofibroblastic features (Fig. 5F). They are less common that GISTs amounting no more than 10 - 15% of GI mesenchymal tumors at different sites.

Metastatic melanoma involving the intestines can sometimes simulate a GIST, as these tumors are often amelanotic. This is especially true for large metastases with significant intramural involvement. Relatively common KIT-positivity of metastatic melanoma (40 - 40%) can increase the potential for confusion [80, 91], as can variably spindle and epithelioid morphology of melanoma (Fig. 5G). The positivity for other melanoma markers, such as HMB45, melanA and tyrosinase is helpful in the diagnosis of melanoma, because these markers are absent in GISTs in our experience.

Malignant epithelial tumors involving the stomach and intestines can sometimes form a GIST-like mass. We have experienced this occurrence in the following isolated instances: a primary undifferentiated carcinoma of the small intestine or colon with pleomorphic and spindle cell features, an esophageal spindle cell squamous carcinoma, and gynecological carcinosarcoma (malignant mixed muellerian tumor) involving the external surface of small intestine. The latter case was KIT-positive, but formed inconspicuous glands highlighted with keratin immunostaining.

Lymphohematopoietic tumors rarely enter in the differential diagnosis of GIST. In practice, we have experienced true histiocytic lymphoma sometimes forming a GIST-like intestinal mass (Fig. 5H). Immunohistochemically, these tumors are KIT-negative and express histiocytic markers, such as CD68 and CD163.

KIT Gene and Protein and Their Alterations in GIST

KIT gene, mapped to 4q12, encodes a 145 - 160kDa protein, a transmembrane tyrosine kinase receptor (RTK) for stem cell factor (SCF, previously also called Steel factor). KIT displays extensive homology with other members of RTK type III family, such as platelet-derived growth factor receptors (PDGFR), colony-stimulating factor-1 receptor (CSF1R) and FMS-related tyrosine kinase 3 (FLT3).

The type III tyrosine kinase receptor family is characterized by extracellular/ligand-binding domain with five Ig-like loops. The extracellular and cytoplasmic domains are connected by a transmembrane region. The cytoplasmic domain consists of juxtamembrane and tyrosine kinase domains. The latter is divided into an adenosine triphosphate (ATP) binding (tyrosine kinase I) region and a phosphotransferase (tyrosine kinase II) region by a hydrophilic kinase insert [3, 101]. The juxtamembrane domain is important in the autoregulation of RTK receptor phosphorylation [101].

Activation of KIT by its ligand SCF leads to downstream phosphorylation of substrate proteins and subsequently activates networks of signal transduction pathways which regulate important cell functions including proliferation, apoptosis, chemotaxis and adhesion. KIT expression is critical for the development and maintenance of mast cells, hematopoietic stem cells, melanocytes, gametocytes, and ICCs in the GI tract [9, 14, 61, 130, 133, 136].

Overview of KIT mutations in GISTs

Somatic (non-inheritable) activating mutations in KIT gene are considered to be a major driving force in the pathogenesis of sporadic, non-familial GISTs. In general, these mutations are believed to activate (phosphorylate) KIT independent of the ligand binding signal [40, 42]. This has been specifically shown of some mutation types, which have been shown to be transforming or leading to KIT autophosphorylation [42, 64, 69, 95]. Most of the KIT mutations have been heterozygous in nature, consistent with the concept of a dominant oncogene activated by monoallelic mutation. Structurally similar, constitutional, inheritable germline mutations have been found in patients with familial GISTs [97].

In GISTs, the majority of KIT mutations have been identified in the juxtamembrane domain, exon 11 [42]. In addition, mutations in the extracellular (exon 9) and tyrosine kinase domains (exons 13, 14 and 17) have also been reported in a small number of cases [2, 55, 68, 107]. However,

a recently reported deletion of Ser^{715} (exon 15) in the kinase insert [2] has been shown to represent a KIT splicing event [31, 56], previously described in myeloid leukemia cells [19]. The schematic distribution and types of KIT mutations in GISTs are shown in figure 6. For comparison, data on other human and canine tumors with documented KIT mutations are also displayed. These tumors include acute myeloid leukemia [94], primary myelofibrosis and chronic myelogenous leukemia [36], mast cell leukemia/mastocytosis, sinonasal NK/T-cell lymphoma [65, 66] and seminoma [102, 121].

Generally only one type of KIT mutation is identified in any given tumor, and the presence of two different productive mutations in a GIST has been reported only twice [2, 111]. Also, coexistence of missense and silent KIT mutations was reported in GISTs once [109]. These seem to be extremely rare events, since they have not been reported in large studies [54, 120]. However, a similar coexistence of different KIT mutations in the second KIT tyrosine kinase domain was recently reported in primary mediastinal seminoma [102].

We have not seen true silent mutations in 300 GISTs analyzed for the KIT mutations in exons 9, 11, 13 and 17. However, apparent nucleotide substitution were seen in 3 cases, but were not reproducible on the same DNA template. Such PCR artifacts mimicking point mutations have been reported in PCR-based mutation analysis of DNA from FFPE tissues [131].

Type of KIT mutation may have an impact of Imatinib treatment. *In vitro* experiments and preliminary clinical data suggest that GISTs with KIT mutations affecting extracellular and tyrosine kinase domains may not respond to tyrosine kinase inhibitors equally well as do tumors with mutated KIT juxtamembrane domain [25, 67, 70].

Mutation in KIT juxtamembrane domain (exon 11)

The juxtamembrane domain encoded by exon 11 is the most common region involved by KIT mutations in GIST. This portion just inside the cell membrane is a helical domain of KIT which is believed to functionally represent an inhibitory element regulating the KIT autophosphorylation in response to growth factor signal by SCF [40, 101]. Mutations in the KIT juxtamembrane domain lead to constitutive KIT phosphorylation and have been shown to be transforming in murine lymphoblast cell lines *in vitro* [42, 95].

Mutations in KIT juxtamembrane domain (exon 11) were the first ones described in GISTs. Hirota et al. [42] reported four deletions and point mutation in five of six analyzed tumors.

Fig. 5. Tumors that enter in the differential diagnosis of GIST. A. Intestinal leiomyosarcoma showing fascicles of well-differentiated smooth muscle cells. B. Gastric schwannoma with a peritumoral lymphoid cuff and relatively low cellularity. C. Inflammatory fibroid polyp contains a mixture of oval lesional cells and eosinophils. D. Inflammatory myofibroblastic tumor is composed of large tumor cells with amphophilic cytoplasm and prominent nucleoli. Significant lymphoplasmacytic infiltration is typical. E. Desmoid tumor contains fascicles of fibroblasts in a collagenous background, and mildly dilated vessels are prominent. F. Undifferentiated sarcoma with a spindle cell pattern; this diagnosis is made by exclusion and is based on immunohistochemistry. G. Metastatic melanoma can have epithelioid or spindle cell pattern and can simulate a GIST. H. Intestinal true histiocytic lymphoma contains large, epithelioid tumor cells.

KIT Domain	Exon	Codon	Type of Mutation	Human	Canine
Extracellula	2	52	Missense	PMF, CML	
	8	419	Deletion, Insertion	AML	
	9	502-503	Insertion/duplication	GIST	
Transmembrane	10	530	Missense	AML	
Juxtamembrane	11	Spectrum	In-frame deletion Missense Insertion/duplication	GIST SNK/T-cell Lymphoma Familial GIST	Mastocytoma GIST Mastocytoma
Tyrosine Kinase I	13	642	Missense	GIST Familial GIST SNK/T-cell Lymphoma	
	14	704-705	Deletion	GIST	
Tyrosine Kinase II	17	816	Missense	Mastocytoma Seminoma Mediastinal Seminoma SNK/T-cell Lymphoma	
		818	Missense	Mediastinal Seminoma	
		820	Missense	Mediastinal Seminoma Familial GIST Mastocytoma	
		822	Missense	Mediastinal Seminoma GIST	
		825	Missense	SNK/T-cell Lymphoma	

Fig. 6. Diagram of the distibution of KIT mutations in GISTs and other tumors. Abbreviations: PMF=primary myelofibrosis, AML=acute myeloid leukemia, CML=chronic myeloid leukemia, SNK/T lymphoma=sinonasal killer/T-cell lymphoma.

Subsequently, large numbers of GISTs have been analyzed confirming that KIT exon 11 is the region most commonly altered by mutations in GIST [30, 54, 109, 120].

On the genomic DNA level, three mutation types have been identified in KIT juxtamembrane domain: in-frame-deletions, missense mutations and insertion/duplications. These mutations alone or in combination modify KIT protein in frame by deleting, replacing or adding amino acids. Examples of different mutation categories and modified KIT proteins are shown in figure 7.

A great majority of exon 11 mutations are in-frame-deletions of one to several codons. Typically these mutations cluster between Lys^{550} and Glu^{561}, most commonly involving Trp^{557} and Lys^{558}. Occasionally deletions extend distally involving large portion of exon 11 and eliminating almost two thirds of KIT juxtamembrane domain [Lasota et al., unpublished observations].

In-frame-deletions in the distal part of exon 11 are seen less frequently, however, their functional significance appears to be similar to that of the typical mutational "hotspot". For example, deletion of Asp^{579} was shown to activate KIT protein [95].

Missense mutations in exon 11 have been reported in 10 - 15% of cases [107, 120]. A great majority of these point mutations clustered in the 5'-end of the juxtamembrane domain and lead to substitution of Asp for Val in codons 559 and 560 [107, 120, Lasota et al., unpublished observations]. Occasionally, a C to T point mutation at codon 576 has been reported in 3'-end of KIT juxtamembrane domain [35, 54, 107, 129]. Identical mutations substituting Pro for Leu^{576} was documented in canine GISTs [33] and canine mastocytoma, where this type of mutation has been shown *in vitro* to cause ligand-independent autophosphorylation of KIT protein [69].

A majority of insertion mutations in the KIT juxtamembrane domain represent tandem duplications of one to several codons. Such internal tandem duplications (ITDs) are relatively rare and occur almost exclusively in 3'-end of KIT juxtamembrane domain [88, 92, 107, 129]. However an insertion of Pro distal to missense mutation substituting Pro for Val^{558} in the 5'-end of KIT juxtamembrane domain, has also been reported in a rare cases [2, 120, 129].

Large ITDs affecting 3'-end of juxtamembrane domain have also been reported in canine mastocytoma and shown to constitutively phosphorylate KIT protein [64, 70, 135]. Interestingly, similar ITDs have been found in the juxtamembrane domain of another type III RTK gene, Flt-3 in acute myeloid leukemia [53, 93].

KIT Exon 11 mutations have been reported in GISTs from different locations from esophagus to anus [88]. Some of the early studies reported that KIT exon 11 mutations are more common in large and malignant GISTs [54, 120] and adverse prognostic significance of such mutations was suggested [30, 54, 120]. However, others [18] have also shown these mutations in diminutive, clinically indolent incidental tumors. Also, site
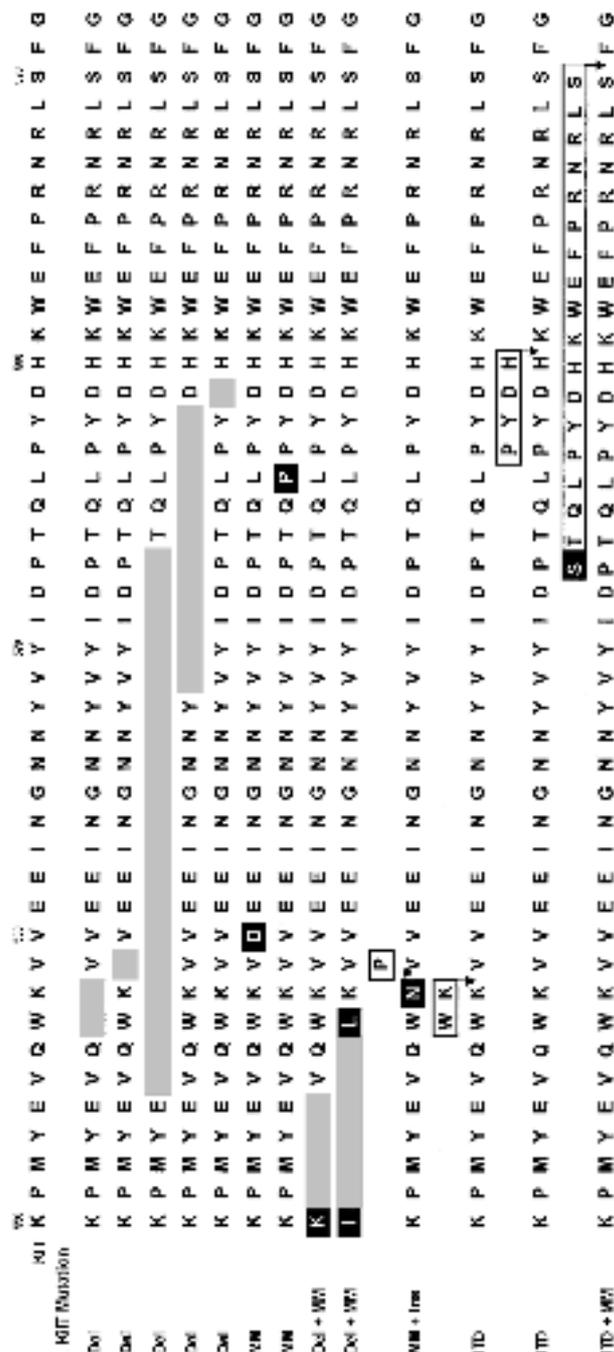
18

Fig. 7. Examples of different KIT juxtamembrane domain mutations in GISTs. Gray, black and clear boxes indicate in-frame-deletions, missense mutations and insertions/internal tandem duplications, respectively. KIT wild type sequence and codon numbers are shown above. Abbreviations: Del-deletion, PM-point mutation, Ins-insertion, ITD-internal tandem duplication, MM-missense mutation.

specific studies, although pooling different mutations together, have not shown the presence of KIT mutations as a statistically significant adverse factor [79, 83, 89]. More recently, Singer et al. [118] suggested that tumors with missense mutations in exon 11 have longer recurrence-free survival than GISTs with other type of KIT mutation. How-

ever, this study was based on relatively small number of cases and should be interpreted with caution. Therefore, prognostic significance of KIT juxtamembrane mutations in GIST is still unclear.

Frequency of the KIT juxtamembrane mutations varies between different studies from as low as 25% to as high as 92% of cases [92, 107]. Several factors may contribute to these differences. Studies based on nucleic acids obtained from fresh/frozen GISTs can show a higher frequency of mutations than one based on partially degraded DNA from FFPE tumors, because some of the KIT mutations, for example, large ITDs may not be amplifiable from partially or severely degraded DNA. Selection of the tumors can also be a factor, because GISTs from certain sites or histological subtypes may differ in KIT mutational status. For example, recent studies have suggested that gastric epithelioid GISTs previously known as leiomyoblastomas may be more often mutation negative [129]. Finally, ethnic differences between study populations should also be considered. None of 172 GISTs including 122 gastric cases studied in Japan [109, 120] revealed large ITDs in distal part of exon 11. In contrast, the frequency of this type of KIT mutation in Western population varies from 3 to 6% for GISTs from different locations reaching a 6 - 9% frequency in gastric GISTs [107, Lasota et al, unpublished observations].

Mutation in KIT extracellular domain (exon 9)

Exon 9 encodes the distal part of the KIT extracellular domain. This exon is the second most commonly mutated KIT region in GISTs. All reported mutations have been structurally identical duplications of six nucleotides, GCC TAT, encoding Ala^{502}-Tyr^{503}. This mutation was first reported by Lux et al. in 8 out of 13 GISTs negative for exon 11 mutations [68].

Subsequent studies have shown Ala^{502}-Tyr^{503} duplication in approximately 5% of GISTs from mixed locations and its strong predilection to intestinal tumors [55]. Only isolated gastric GISTs carrying this type of KIT mutation have been reported [44, 111, 129]. A great majority of GISTs with KIT exon 9 mutation are clinically malignant [4, 55]. The response of tumors with this mutation to tyrosine kinase inhibitor treatment is being investigated.

Other segments of KIT extracellular domain (exons 1 to 9) have also been found mutated in myeloproliferative disorders and acute myeloid leukemia [36, 94].

Mutation in KIT tyrosine kinase I domain (exon 13 and 14)

A rare missense mutation resulting in substitution of Glu for Lys^{642} in exon 13 encoding part of the tyrosine kinase I, the ATP-binding domain of KIT, was initially reported in 2 GISTs negative for the mutation in exon 11 [68]. Subsequent studies of 200 and 48 tumors [55, 111] estimated the frequency

of this mutation to be no higher than 1%. Based on small number of cases, this mutation is associated with malignant behavior [55].

Homozygous exon 13 mutations have been found to lead into constitutive KIT tyrosine phosphorylation [68]. A GIST cell line with this mutation was found to be sensitive to Imatinib, which also abolished the phosphorylated status of KIT [126].

More recently, an in-frame-deletion of two codons, Lys^{704} and Asn^{705} in exon 14 in the distal part of the KIT tyrosine kinase I domain, was reported in a GIST with an in-frame-deletion of codons 568-560 in exon 11 [2]. However, no mutations were found in KIT exon 14 in a subsequent study of 31 GISTs suggesting that this type of mutation must be a rare event if it occurs [56].

Mutation in KIT tyrosine kinase II domain (exon 17)

Missense mutations substituting Val for Asp^{816} in the KIT tyrosine kinase II domain (exon 17) were the first to be identified in KIT associated mastocytosis and urticaria pigmentosa [65, 66]. This mutation has been shown to cause ligand-independent autophosphorylation of KIT. Similar missense KIT mutations were found in human gonadal germ cell tumors of seminoma/dysgerminoma type [121], mediastinal seminomas [102] and sinonasal natural killer/T-cell lymphomas. The latter also showed missense mutations affecting KIT juxtamembrane domain [46].

No exon 17 mutation was found in an initial large study of 124 GISTs [120]. However, subsequently, 2 GISTs with point mutations leading to substitution of Lys or His for Asp^{822} were reported [107]. In our series of over 100 GISTs screened for exon 17 mutations, only two cases were positive [Lasota, unpublished observations]. These rare mutations appear to confer resistance to tyrosine kinase inhibitor treatment [70].

Alternative receptor tyrosine kinase mutations in PDGFRA

Approximately 35% of GISTs negative for KIT mutations were recently shown to have activating mutations in the PDGFRA gene encoding the platelet derived growth factor alpha leading to similar signaling consequences as KIT mutations did [41]. Notably, tyrosine kinase inhibitor Imanitib also inhibits PDGFR kinase [25].

Other Genetic Changes and Their Pathogenetic Role in GISTs

KIT mutations are believed to be major genetic event leading to GIST tumorigenesis. However, a recent study based on HUMARA (human androgen receptor assay) showed that diffuse proliferations of Cajal cells in a patient with familial GISTs represented polyclonal non-neoplastic hyperplasia, whereas their GISTs were monoclonal [15].

This suggests that the growth of a GIST requires additional genetic changes, a so-called "second hit".

Karyotyping, molecular cytogenetic and molecular genetic studies have commonly shown losses of chromosomes 14q, and 22q in benign and malignant tumors indicating that these losses are early changes in GIST tumorigenesis [6, 11, 21, 23, 26, 37]. Deletion mapping studies have defined the regions on chromosomes 14 and 22 that may harbor putative tumor suppressor genes, but no specific genes have been identified [22, 23, 27]. Study of neurofibromatosis type 2 (NF2) tumor suppressor gene, mapped to 22q12, in a small series of GISTs infrequently showed mutations giving no conclusive evidence of NF2 gene involvement in GIST pathogenesis [34].

Losses of chromosomes 1p, 9p and 15 have also been documented in a subset of GISTs and linked to aggressive tumor behavior [28, 37]. Alterations of cyclin dependent kinase 4 inhibitor (P16INK4) gene mapped to 9p21, initially documented in a small number of malignant GISTs [49], were found to be an independent prognostic marker in a more recent study [117]. Also losses in the short arm of chromosome 1, especially involving band 1p36, seem to correlate with poor prognosis, according to one study [98].

Study of benign and malignant GISTs by comparative genomic hybridization (CGH) has shown gains and amplifications of DNA copy numbers in 5p, 8q, 17q and 20q predominantly in malignant GISTs and especially in metastatic tumors. Although no amplified genes have been specifically identified in these regions, CGH evaluation could have a prognostic predictive value [28].

Future Prospects

Further delineation of malignant potential by GISTs by detailed clinicopathologic, biological, and genetic studies will continue to give more accurate information in relation to optimization of treatment, especially that with tyrosine kinase inhibitors. The high cost and potential long term side effects of the new treatment necessitate careful patient selection, including clinicopathologic identification of tumors that will have a favorable course without such a treatment. Also correlation of molecular genetics and treatment results and evaluation of resistance to tyrosine kinase inhibitors would be important. Evaluation of KIT signaling pathways in GISTs with different KIT mutations and identification of gene expression profiles by cDNA microarray studies [1] and proteomics is also emerging. Study of additional genetic changes could reveal pathogenetically critical events.

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