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Pathology and diagnostic criteria of gastrointestinal stromal tumors (GISTs): a review [1]

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Abstract

Gastrointestinal stromal tumor (GIST) is the designation for the specific c-*kit* expressing and Kit-signaling driven mesenchymal tumors, many of which have Kit-activating mutations. The specific identification of GIST has become increasingly important because a Kit-selective tyrosine kinase inhibitor, imatinib (Glivec®, formerly known as STI571, Novartis Pharma AG, Basel, Switzerland), has shown promise as an effective adjuvant therapy treatment. GISTs are the most common mesenchymal tumors of the gastrointestinal (GI) tract. We estimate the frequency of malignant GISTs as 20% to 30% of the frequency of all soft-tissue sarcomas, but small benign tumors, often found incidentally during unrelated surgery or autopsy, are probably much more common. Older adults are most at risk for GIST; very rarely, GIST occurs in children and young adults (sometimes connected with Carney's triad), or on a familial basis. GISTs have been documented in all parts of the GI tract. A great majority of them occur in the stomach (60% to 70%) and small intestine (25% to 35%), with rare occurrence in the colon and rectum (5%), esophagus (<2%) and appendix. Some GISTs are primary in the omentum, mesentery or retroperitoneum, and are unrelated to the tubular GI tract. GISTs can be histologically identified as highly cellular spindle cell or epithelioid mesenchymal tumors, and morphology is somewhat site-dependent. However, common to all these tumors is expression of Kit (CD117 antigen), which is a major diagnostic criterion. Few other Kit-positive mesenchymal tumors of the GI tract are likely to be confused with GISTs; exceptions are metastatic melanoma and related tumors and malignant vascular tumors. Additional diagnostic criteria include common positivity for CD34 (70%), variable expression of smooth muscle actins (20% to 30%) and S100 protein (10%) and almost uniform negativity for desmin (only 2% to 4% of GISTs are positive). Although the prediction of malignancy in this tumor group is notoriously difficult, tumors that have mitotic activity counts exceeding 5 per 50 high power fields (HPF) or those larger than 5 cm have a high frequency of intra-abdominal recurrence and liver metastasis. In contrast, tumors smaller than 2 cm and those with mitotic activity counts <5 per 50 HPF are likely to be benign. These diagnostic criteria leave an inevitable gray area in the separation of benign and malignant tumors. Kit-activating mutations can be detected in at least 60% to 70% of GIST cases. Most of the mutations, in-frame deletions of several codons, are located in the juxtamembrane domain (exon 11) of the gene. Less commonly, mutations have been detected in the extracellular domain (exon 9), and tyrosine kinase domains (exons 13 and 17). Functional analysis of the different c-*kit* mutations and their impact on the response to tyrosine kinase inhibitors are under intense investigation.

Keywords: GISTs; KIT; Mutations

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1. Introduction

Gastrointestinal stromal tumor (GIST) is the name applied to a group of Kit-positive mesenchymal tumors specific to the gastrointestinal (GI) tract and abdomen. Pathologic activation of Kit signal transduction is believed to be a central event of GIST pathogenesis [1,2]. The identification of GIST has become very important since specific, pathogenesis-targeted treatment with Kit tyrosine kinase inhibitor, imatinib (Glivec®, formerly known as STI571, Novartis Pharma AG, Basel, Switzerland), has become available and shows promise in metastatic GIST [3]. Two series from clinical trials showing favorable results in metastatic or unresectable GISTs were also recently presented at the annual meeting of the American Society of Clinical Oncology [4,5].

This review will discuss the pathology and diagnostic criteria of GISTs. These neoplasms comprise a vast majority of the tumors that were formerly almost always diagnosed as leiomyomas, leiomyosarcomas, leiomyoblastomas and smooth muscle tumors of the GI tract and adjacent abdominal sites, with a few exceptions (Table 1) [1,2,6–10]. Gastrointestinal autonomic nerve tumors (GANTs), previously defined as a separate entity, are now understood to be ultrastructural variants of GIST [11]. They are histologically similar to GISTs, are Kit-positive and have GIST-specific c-*kit* mutations [12].

Since the majority of mesenchymal tumors of the GI tract (except those in the esophagus and muscularis mucosae of the colon and rectum) are GISTs, older data pertaining to GI smooth muscle tumors [6–10] should actually be categorized as data concerning GISTs.

2. Definition and diagnostic features of GIST

GISTs are defined here as cellular spindle cell, epithelioid or occasionally pleomorphic mesenchymal tumors of the GI tract that express Kit protein (Fig. 1). As detected by immunohistochemistry, the majority of GI mesenchymal tumors are GISTs and are strongly and nearly uniformly Kit-positive [13–15].

Relatively few other tumors are variably or consistently Kit-positive, and these tumors only rarely enter in the differential diagnosis of GISTs. Such neoplasms include metastatic melanoma, clear cell sarcoma, Ewing family tumors, neuroblastoma, angiosarcoma, mastocytoma, blastic extramedullary myeloid tumor (tissue manifestation of acute myeloid leukemia), seminoma, pulmonary small cell carcinoma and some other carcinomas [16–20].

The GIST definition shown above excludes GI true smooth muscle tumors, such as esophageal leiomyomas [21,22] small colorectal leiomyomas of the muscularis mucosae [23], and rare GI true leiomyosarcomas [22,24,25], each of which have distinctive clinicopathologic features (Table 2). Also excluded are glomus tumor [26], schwannoma [27,28], inflammatory fibroid polyp [29,30], inflammatory myofibroblastic tumor [31,32] and abdominal (dedifferentiated) liposarcoma [33], whose clinicopathologic features are also summarized in Table 2. Although these

Table 1
Extrapolation from the previously employed diagnostic terminology to current terminology related to GISTs

Previous terminology	Current terminology and comment
Esophageal leiomyoma	Most of these tumors are true leiomyomas, histologically and clinically separate from GISTs
	Esophageal leiomyosarcomaMost of these tumors are GISTs, and a small minority are true leiomyosarcomas
Gastric leiomyoma	The great majority are GISTs; 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
Leiomyoma/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, omentum, mesentery and retroperitoneum. Clinical and gross pathology correlation is needed to determine the primary site

Table 2
Kit-negative GI tumors that may resemble GISTs clinically or pathologically (all types immunohistochemically tested by authors)

Tumor entity	Similarities to and differences From GIST
Esophageal leiomyoma [21,22]	Intramural esophageal tumor composed of well-differentiated, actin- and desmin-positive smooth muscle cells. Much less cellular than GIST. Occurs more often in young patients than GIST
Pericolonic leiomyoma [21]	Occurs in women; histologically similar to uterine leiomyomas. Positive for estrogen and progesterone receptors, actin and desmin
True leiomyosarcoma [22,24,25]	A rare subset of spindle cell sarcomas (up to 10%) that show phenotypic features of well-differentiated smooth muscle cells and often manifest as polypoid intraluminal masses, typically in older adults
Glomus tumor [26]	Identical to glomus tumor of peripheral soft tissue. Occurs almost exclusively in the stomach in the GI tract Positive for smooth muscle actin, negative for desmin. May be variably CD34-positive; only mast cells Kit-positive
Inflammatory fibroid polyp [29,30]	Spindle cell lesion, may be CD34-positive. Slender spindle cells admixed with lymphoid cells and eosinophil granulocytes. Some variants are highly vascular and granulation tissue-like with a loose texture. They have a greater cellular heterogeneity than GISTs do. Grossly, these lesions often represent ulcerated intraluminal polyps
Inflammatory myofibroblastic tumor (IMT) [31,32]	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
Mesenteric desmoid	May have a GIST-like gastric or intestinal wall involvement. Grossly very firm and white. Fibroblasts and myofibroblasts in collagenous background. CD34-negative
Solitary fibrous tumor	May present on the peritoneal surfaces or in the liver. Collagenous spindle cell tumor, with a focal hemangiopericytoma-like pattern. CD34-positive
Schwannoma [27,28]	Usually a small, 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. GFAP positivity is also common; this is almost never seen in GISTs
Undifferentiated sarcomas	Malignant GI tumors, which do not express any specific cell-type markers and cannot currently be further defined. May grossly simulate GISTs, but histologically often show greater nuclear pleomorphism than GISTs
Dedifferentiated liposarcoma [33]	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, GIST forms a polypoid intramural lesion. Positivity for melanocytic markers (tyrosinase, melanA, HMB45, in various combinations), is diagnostic

tumors are almost invariably Kit-negative, some tumors other than GISTs (such as typical leiomyosarcomas and liposarcomas) may contain isolated Kit-positive cells [18]. Considering that GISTs are typically globally Kit-positive, the sporadic Kit-positive cells in other tumors are not likely to be confusing in the differential diagnosis. However, from the treatment perspective, some non-GIST Kit-positive tumors may be potential candidates for treatment with Kit tyrosine kinase inhibitors.

3. Tumors that should be evaluated for the diagnosis of GIST

It is important to specifically identify GISTs because of the availability of the Kit tyrosine kinase inhibitor imatinib, which has shown promising clinical results [3]. Immunohistochemical evaluation for Kit (CD117) to examine the possibility that the tumor is a GIST should be considered at least in the following instances:

1. Primary mesenchymal tumors of the tubular GI tract, with the possible exception of typical esophageal leiomyomas, small polypoid leiomyomas 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 mesenchymal tumors of the abdomen and abdominal wall
4. Spindle cell tumors of the liver

4. Evaluation of kit positivity

The ability to reach the correct diagnosis of GIST is critically dependent on the pathologist's familiarity with the histologic spectrum of GIST and the use of well-documented Kit antibodies and immunostaining techniques. The primary identification of GIST should take place in the hospital managing the original pathology, since the patient may never enter into treatment consideration if the diagnosis of GIST is initially missed. Diagnostically difficult cases may benefit from consultations with specialized centers. In treatment trials, confirmation of GIST diagnosis is often assigned to a centralized pathology panel; at this stage, non-GIST patients who may not benefit from the GIST-specific treatment may be assigned to other types of treatment.

Normal Kit-positive cells in abdominal soft tissues include mast cells present in the wall of the GI tract (especially in the submucosa) and the slender, spindled interstitial cell of Cajal present around the myenteric plexus and in parts of the muscularis propria [13–16]. The normal Kit-positive components are important positive controls to validate the quality of the immunostaining, especially the adequacy of heat-induced epitope retrieval, whether based on microwave, steamer or autoclave heat. One such mode of epitope retrieval must be used for successful Kit immunostaining. Fibroblasts and normal smooth muscle cells should be Kit-negative. If an avidin-biotin-based detection system is used, avidin-biotin block is necessary to eliminate the detection of endogenous biotin. For example, this is present in hepatocytes, some other epithelial cells and, importantly, in some tumor cells, and can cause false-positive staining.

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. In our experience, some epithelioid GISTs of the stomach are less uniformly positive (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. According to our experience, the best ones currently available for formalin-fixed and paraffin-embedded tissue are polyclonal antibodies. The monoclonal antibodies currently available react inconsistently in formalin-fixed and paraffin-embedded tissue and identify only a minority of GISTs.

5. Other differentiation markers in GISTs

Approximately 70% to 80% of GISTs are positive for CD34, a hematopoietic progenitor cell antigen also present in endothelial cells and subsets of fibroblasts and many neoplasms related to these cell types [34–37]. GISTs of the esophagus and rectum are more consistently CD34-positive than are the gastric and small-intestinal GISTs. There seems to be no difference in the frequency of CD34 expression between benign and malignant GISTs [18].

Approximately 30% of GISTs, especially gastric and small-intestinal tumors, are positive for smooth muscle actin, whose expression tends to be 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.

S100 protein expression is relatively rare in GISTs, and occurs most commonly in the small intestine (10%). The positivity is usually focal, but is present in both cytoplasm and nuclei, and likely represents true expression of this antigen.

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 [22]. 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 immunophenotyping.

The embryonic form of smooth muscle myosin [38] and heavy caldesmon, an actin-binding cytoskeletal protein [39], are smooth muscle antigens frequently expressed in GISTs; their expression suggests that these tumors may be related to smooth muscle precursor cells. Recently, GISTs were reported to be consistently positive for nestin, a type VI intermediate filament protein. Experience with this marker is limited, but nestin is also expressed in other tumors, such as rhabdomyosarcomas and melanomas [40].

Like most mesenchymal tumors, especially those that are malignant, GISTs are positive for vimentin. Keratin positivity, in our experience, is rare (approximately 10% of cases), and can be seen with antibodies reacting to keratin 18 and, to a lesser degree, to keratin 8.

6. Significance of the phenotypic variants

Previously GISTs were sometimes divided into tumors of myoid, schwannian-like or mixed phenotypes based on their antigen expression patterns (e.g., actins or S100). However, the earlier subclassifications did not accurately address the differential diagnosis of GIST, leiomyoma and schwannoma in modern terms, but included several unrelated entities under GIST. Separate from this, GIST was also sometimes used as an umbrella term for different types of GI mesenchymal tumor.

The new understanding of the role of Kit activation as a common factor in all GISTs lessens the apparent clinical significance of the phenotypic variants. However,

the possible significance of such variants of GISTs (CD34-positive, S100-positive and actin-positive GISTs) needs to be evaluated in large clinicopathologic series.

7. Histologic features

GISTs show a spectrum of cellular patterns, including spindle cell, epithelioid and, rarely, pleomorphic. Examples of the histologic spectrum of Kit-positive GISTs are shown in Fig. 1.

The spindle cell GISTs are typically highly cellular tumors, often having an overall basophilic appearance because of high nuclear density and relatively scant cytoplasm. This is especially true when GISTs are compared with typical leiomyomas, such as those of the esophagus and of the muscularis mucosae of the colon and rectum. The nuclei of GIST cells tend to have relatively pointed ends (as compared with blunt-ended nuclei in the typical leiomyosarcomas). Nuclear palisading and bundling of tumor cells in fascicles separated by myxoid stroma and perinuclear vacuolization are common features of GIST. Many small intestinal GISTs and some colonic GISTs, especially the nonmalignant tumors, are distinctive for their microscopically distinctive, round aggregates of extracellular collagen fibers, which have a skein-like ultrastructural appearance and have therefore been named skeinoid fibers [1,41,42].

The epithelioid GISTs most commonly occur in the stomach, where they comprise approximately one third of all GISTs, and correspond to the previous designation of leiomyoblastoma. These tumors also occur in the omentum and as disseminated intra-abdominal tumors of undefined origin. They are typically composed of polygonal cells with ample, amphophilic cytoplasm and round nuclei. Focal nuclear pleomorphism is common in epithelioid GISTs, and some of these tumors have an organoid paraganglioma-like compartmental pattern.

A small minority of GISTs (<3% to 5%) have extensive nuclear pleomorphism; most abdominal mesenchymal tumors with marked pleomorphism are not GISTs but, rather, are pleomorphic leiomyosarcomas or undifferentiated and unclassified malignant mesenchymal tumors, some of which are currently being classified as malignant fibrous histiocytomas.

8. Epidemiology of GIST

According to large clinicopathologic series, GISTs predominantly occur in individuals over 40 years of age, with the median ages in the largest series of GISTs of different sites varying between 55 and 65 years [1]. Some series show a male predominance and others show an equal sex distribution. GISTs are very rare in children; we have found that many tumors classified earlier as GI smooth muscle tumors in children actually were inflammatory myofibroblastic tumors.

Based on a population-based sample from Southern Finland, we originally estimated the incidence of malignant GIST to be 4 per million and the total incidence to be approximately 40 per million [43]. This estimate is probably too low, since many tumors not originally classified as GI primary tumors were not included; on the other hand, the total incidence may not be as high as originally predicted. Nevertheless, the overall incidence of malignant GISTs could represent 20% to 30% of the incidence of all soft tissue sarcomas. The prevalence of GISTs is much higher, since many malignant tumors have a long clinical course of 5 to 15 years.

Although the incidence of malignant GISTs can be relatively easily determined based on Cancer Registry and Tumor Registry records, the incidence of benign GISTs is much more difficult to establish, and requires systematic, very laborious retrospective analysis of autopsy and surgical pathology files on a regional basis.

GISTs are most common in the stomach (60% to 70%), followed by the small intestine (25% to 35%), the colon and rectum (5%) and the esophagus (<2%) [43]. We have also seen isolated cases in the appendix. GISTs may also occur as primary in the omentum and mesenteries [44] and the retroperitoneum [45]. The number of extragastric GISTs may be larger than originally estimated. For example, we have found that up to one third of archival retroperitoneal leiomyosarcomas may be GISTs [18]. However, close examination of many of these cases reveals definitive or probable tumor origin from the stomach or small intestine. Testing for c-*kit* expression in abdominal mesenchymal tumors is important to ensure that GISTs outside of the confines of stomach and intestines can be diagnosed and specifically treated.

Very rarely, we have seen GISTs as metastatic tumors in the peripheral soft tissues in locations such as the arm and abdominal wall; this can be clinically confusing if history of a previous abdominal tumor is not available.

9. GIST as a part of tumor syndromes

In rare instances GISTs occur as part of tumor syndromes. Carney's triad, described by endocrine pathologist J. Aidan Carney from the Mayo Clinic, includes gastric GIST, paraganglioma and pulmonary chondroma (by definition, at least two of these tumors seen in one patient) [46]. We have seen only a handful of such cases, including a man who developed an epithelioid gastric GIST at the age of 26, local recurrence at age 31 and two paragangliomas (carotid body and vagal tumors) at the age of 51. Our experience indicates that these tumors are Kit-positive and as such are comparable with epithelioid GISTs, corresponding with the previous terms leiomyoblastoma, epithelioid leiomyoma or leiomyosarcoma.

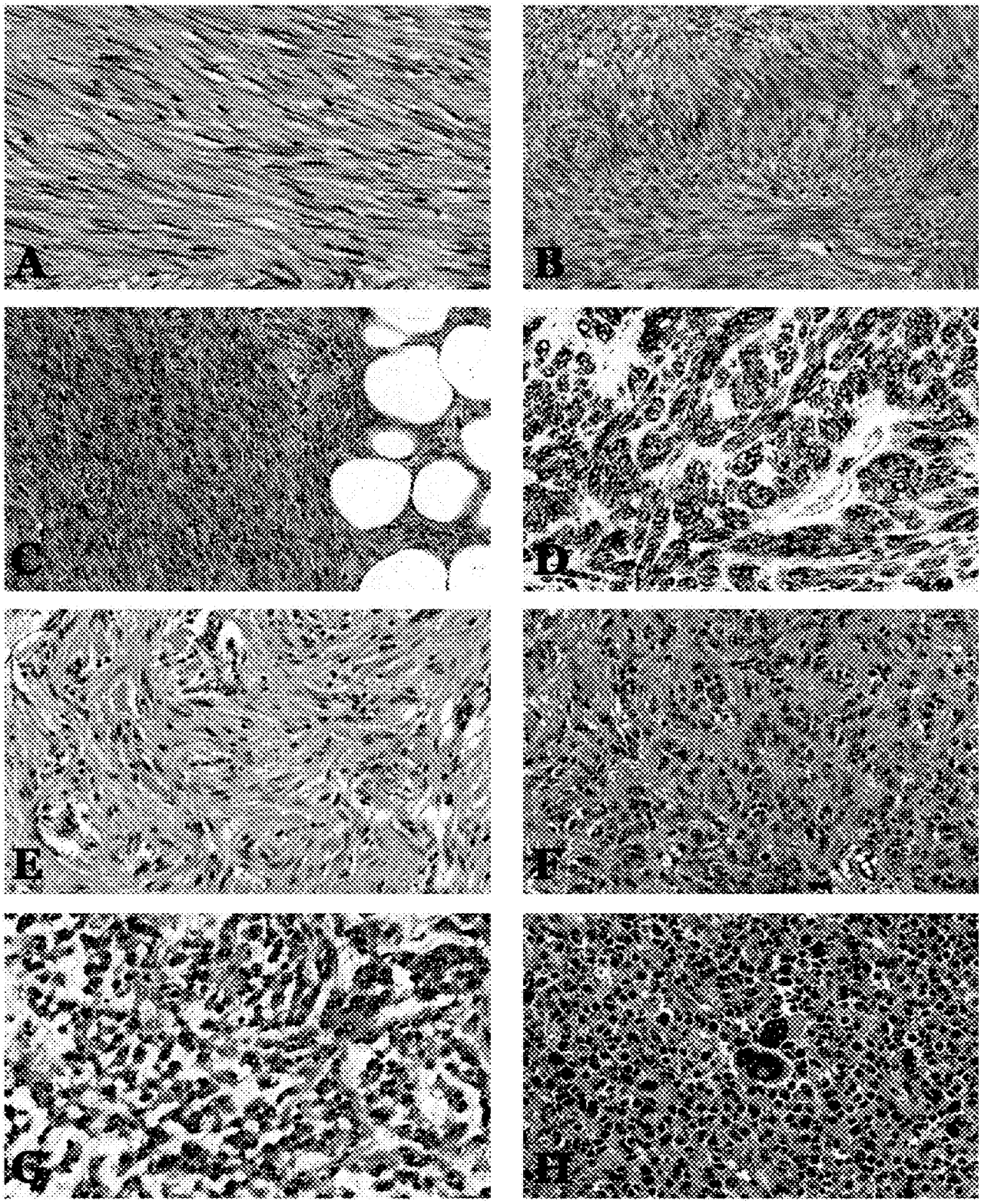

Fig. 1. Histologic spectrum of GIST and examples of Kit immunohistochemistry. A. Benign gastric GIST with only moderate cellularity and no mitotic activity. B. Benign but cellular gastric GIST with nuclear palisading reminiscent of a schwannoma. C. Malignant gastric GIST infiltrating in perigastric fat; this tumor metastasized to liver and peritoneum. D. Strongly positive Kit immunostaining of the case shown in panel C. E.Benign small intestinal GIST with a relatively low cellularity and skeinoid fibers. F. Malignant small intestinal GIST that metastasized to the liver. G. Malignant intra-abdominal GIST of unknown origin showing a trabecular and myxoid pattern. H. Malignant intra-abdominal GIST with an epithelioid pattern and focal pleomorphism of an unknown origin; tumors with such an epithelioid pattern are often of gastric or omental origin.

A recent review by Carney described 79 such patients from the Mayo Clinic and from the literature [46]. The GI tumors were exclusively located in the stomach. stomach and were of indolent nature. The group of patients surveyed had a striking female predominance (85%) and a majority had an indolent behavior. However, while 41% of the patients experienced a local recurrence and many had liver metastases, many survived for long periods of time, even with metastatic disease. Disease-related mortality was only 13%.

GISTs occur in connection with neurofibromatosis type 1 (NF1) syndrome [47], and patients with this combination sometimes have multiple small intestinal GISTs or diffuse Cajal-cell proliferation, an otherwise rare occurrence. A recent study points to a nonrandom association between NF1 and GISTs [48]. The nature of this association and its possible relationship with the NF1 gene alterations in the tumors are unknown.

10. Tumor behavior and prognostic factors at different sites

Study of site-specific and combined series of GISTs indicates that these tumors have a spectrum spanning from small, benign, usually incidentally detected nodules to overt sarcomas at all sites of occurrence [23–25,49–52]. However, for clinical reasons, the relative frequency of detection of GISTs as small tumors (more common in gastric and rectal GISTs) or advanced sarcomas (more common in small intestine and colon GISTs) varies. We still do not know whether the small GISTs are precursors to sarcomas or represent a biologically indolent subset of GISTs.

Small GISTs are often incidentally detected on the external aspect of the stomach or small intestine during abdominal surgery. GISTs may also be detected at gastroscopy as submucous nodules or occasionally as incidental radiologic findings. Small rectal GISTs are often detected during routine prostate or gynecologic examination. According to our experience, such small tumors are generally clinically harmless lesions. However, small benign solitary GISTs should not be confused with situations where multiple small GIST nodules have disseminated from a malignant GIST.

The symptomatic GISTs of the esophagus typically present with dysphagia, or as a mediastinal tumor connected with the esophagus [22]. According to our experience, most esophageal GISTs are clinically malignant and behave as sarcomas, although a small number of tumors (10% to 20%) are detected as small nodules incidentally and have a good prognosis in follow-up studies [22].

Gastric and small intestinal GISTs often present with vague symptoms leading to their gastroscopic or radiologic detection, but sometimes they cause upper GI bleeding when they have a superimposed mucosal ulceration. Gastric GISTs seem to behave less aggressively than small intestinal tumors of similar size and mitotic activity [21,53].

Colorectal GISTs may manifest with lower GI bleeding, obstruction, perforation, pain or a combination thereof. A majority of them are detected as advanced tumors [23,24].

The most important and easily applicable histologic criteria for prediction are tumor size (maximum diameter in cm), and mitotic rate [49–52]. A rate of $\leq$5 mitoses per 50 HPF is commonly used as a limit for a tumor of expected benign behavior, and according to a large study, it discriminated between benign and malignant gastric tumors, but not between benign and malignant small intestinal tumors [49]. Tumors of the size of 2 cm are generally expected to behave in a benign fashion. For example, in the rectum, such tumors were indolent according to our recent follow-up study of over 100 rectal GISTs [24]. Degree of cellularity and atypia have also been suggested as useful criteria, but their reproducibility is more problematic.

The malignant GISTs (tumors with a rate $>$5 mitosesmitotic counts per 50 HPF) have a high risk for diffuse intra-abdominal spread and liver metastasis, the two most common modes of dissemination of malignant GISTs. Distant metastases to other sites, especially bone and lung, are relatively rare [7,9,24]. Soft-tissue metastases may be seen in the internal aspect of the abdominal wall and occasionally in the subcutis elsewhere; in the latter context, the specific diagnosis may be difficult.

Tumors with a rate $>$50 mitosesmitotic counts per 50 HPF are customarily designated as high-grade malignant. However, by this criterion, only a minority of GISTs are high-grade tumors, and most malignant GISTs are low-grade malignant and run a slow course of disease with recurrences and metastases developing over years — sometimes 10 to 15 years after the primary surgery. Therefore, long-term follow-up is essential for these tumors.

However, among mitotically inactive tumors, there is a small percentage that later metastasize, illustrating that a low mitotic count does not rule out the possibility of malignant behavior [6,10,49]. Therefore, the designation "uncertain malignant potential" applies to a significant number of GISTs; at least a close follow-up is needed in such cases.

Ki67 analogs applicable in formalin-fixed and paraffin embedded tissue (e.g., MIB1 and Ki-S5) may assist in tumor evaluation [52–55]. Tumors with more than 10% of nuclear positivity for Ki-S5 have been shown to develop metastases and to have tumor-related mortality with statistical significance [52].

Overall 5-year survival in two recent large series of malignant GISTs presenting combined data on 200 tumors from the Memorial Sloan-Kettering Cancer Center [56] and 191 tumors from the MD Anderson Cancer Center [57] was 35% and 28%, respectively. However, these patients, seen in two large oncologic hospitals, included many patients referred for local failure or metastasis. The 5-year actuarial disease-specific survival was much better — 54% for patients whose tumors were completely resected [56]. These data offer useful baseline information for assessing

the impact of new therapies, such as the Kit tyrosine kinase inhibitors.

11. Histogenesis of GIST

GISTs share phenotypic similarity with the Kit-positive interstitial Cajal cells of the GI tract. These cells function as pacemaker cells that regulate autonomic intestinal motility, and their development depends on cellular signaling regulated by the Kit protein [12,58,59]. They have the capability to differentiate into smooth muscle if deprived of Kit signaling [60]. Experiments on the development of chimeric birds and mice have also shown that the Cajal cells and smooth muscle originate from a common precursor [61,62]. These data strongly suggest that Cajal cells or subsets thereof represent a multipotential stem cell-like population, which is the logical candidate for GIST histogenesis.

12. c-*kit* mutations in GISTs

Ligand-independent activation of the tyrosine kinase protein Kit due to gain-of-function mutations in the c-*kit* juxtamembrane domain (exon 11) seems to be a central event in GIST pathogenesis, and such mutations were shown to be transforming on murine lymphoblast cell lines in vitro [63,64]. Different types of Kit-activating mutations have been reported in GISTs and other tumors, as summarized in Fig. 2. The other tumors with c-*kit* mutations include seminoma [65] and certain hematopoietic neoplasms, such as chronic myeloproliferative disorder [66], acute myeloid leukemia,leukemia [67] mast cell neoplasia [68,69] and sinonasal NK/T-cell lymphoma [70].

In GIST, Kit-activating mutations cluster frequently in a "hot spot" located in the proximal part of exon 11 between Gln^{550} and Glu^{561}. A majority of them represent in-frame deletions (one to several codons, sometimes extending to the 3' part of the exon 11). However, missense mutations have also been reported in approximately 10% of cases [71].

Mutations in the distal part of exon 11 are seen less frequently, and their functional significance appears to be similar to that of the typical mutational "hot spot". For example, deletion of Asp^{579} was shown to activate Kit protein [72]. Occasionally, insertion/duplication of several codons has been reported [1,73,74]. This type of c-*kit*-juxtamembrane mutation was previously reported in canine mastocytoma and shown to be associated with constitutive phosphorylation of the Kit protein [75,76]. The occurrence of c-*kit*-juxtamembrane domain mutation in 50% to 60% of GISTs was shown in several studies

Fig. 2. Diagram of the distribution of the Kit-activating mutations in GIST and other tumors [63,65–79]. Examples of different c-*kit* juxtamembrane domain mutations in GISTs. Gray, black, and clear boxes indicate in-frame deletions, missense mutations, and insertions/duplications, respectively. Exon 11 of c-*kit* wild-type sequence and codon numbers are shown above. *Frequency of the specific mutations among all c-*kit*-juxtamembrane mutations is based on 174 previously reported c-*kit* mutations [71,81]. Abbreviations: Del: deletion; PM: point mutation; In: insertion; Dup: duplication; MM: missense mutation.

[71,77–79] but both lower [73] and higher [74] mutation frequencies have been reported.

Since up to half of GISTs lack mutations in the juxtamembrane domain, the possibility that activating c-*kit* mutations might occur in other gene domains has been evaluated. c-*kit* mutations have been found in extracellular (exon 9) and tyrosine kinase (exon 13 and 17) domains [74,80]. These mutations were duplications of six nucleotides, encoding Ala^{502}–Tyr^{503} in exon 9 and missense mutations, resulting in substitution of Glu for Lys^{642} in exon 13 and Lys or His for Asn^{822} in exon 17. Homozygous exon 13 mutations were associated with constitutive Kit tyrosine phosphorylation [80]. Mutations in exon 9, 13 and 17 were found in 10 of 14 GISTs (71%) that were negative for exon 11 mutations, and the authors concluded that these mutations cover a substantial balance of those GISTs that lack exon 11 mutations [74]. However, data on frequency of c-*kit* mutations is somewhat contradictory at the present. Three series collectively studied 381 GISTs and confirmed the occurrence of specific duplication of Ala^{502}–Tyr^{503} in exon 9, although the frequency of this mutation was only 5% of all analyzed tumors [81–83]. Exon 13 missense mutations were reported in less than 1% of analyzed cases, collectively in two of 248 GISTs [81,83]. Also, no mutations were found in exon 17 in 124 previously analyzed GISTs [71].

A prognostic significance of c-*kit* mutations was suggested by several clinicopathological studies [71,77,78, 81]. The largest series have shown that c-*kit* mutations in exon 11 are more common in larger tumors, and that the presence of these mutations is an adverse prognostic factor [71]. Also, duplication of Ala^{502}–Tyr^{503} in exon 9 may be a marker for malignant course of disease. The majority (71%) of GISTs carrying this mutation were clinically highly malignant, and more than half (59%) have been reported exclusively in small intestinal tumors [81–83].

Recently, familial GISTs have been reported. Such tumors typically are multiple and occur earlier in life than GISTs in general [84–87]. Molecular genetic studies of the tumors and normal tissues have revealed constitutional mutations either in the c-*kit*-juxtamembrane domain [84–86] or Glu-for-Lys^{642} substitution in exon 13 [87]. In some cases, other manifestations related to an activated Kit pathway were seen. They included urticaria pigmentosa in one family [85] and hyperpigmentation in another [86].

The therapeutic significance of the different mutations is under intense investigation [88,89]. For example, the exon 13 mutation that replaces Glu for Lys^{642} was shown to be sensitive to imatinib, which abolished the phosphorylated status of Kit in a cell line [90].

13. Cell signaling in the pathogenesis of GISTs

The Kit protein (CD117 antigen, in the standardized terminology of cluster of differentiation of leukocyte antigens) is a transmembrane growth factor receptor for stem cell factor (SCF), and is therefore also called an SCF-receptor. Human c-*kit* is expressed on mast cells, hematopoietic stem cells, melanocytes, gametocytes and interstitial cells of Cajal, and has been shown to be functionally important for the development and maintenance of these cell populations [91].

The 145–160 kDa gene product is a transmembrane type III tyrosine kinase receptor, and displays extensive homology with other members of this tyrosine kinase receptor family, such as platelet-derived growth factor receptor (PDGFR) and colony-stimulating factor-1 (CSF-1). Kit consists of an extracellular region, a single membrane-spanning region and a cytoplasmic region. The intracellular kinase domain is interrupted by a hydrophilic kinase insert sequence that divides the kinase domain into an adenosine triphosphate (ATP) binding region and a phosphotransferase region. Evidence suggests that activation of Kit by its ligand SCF is critical for diverse mast cell functions, including proliferation of bone marrow-derived mast cells, chemotaxis and cytoskeletal rearrangement [92–97].

The mechanism by which SCF initiates diverse cellular responses results in part from the wide array of signaling-transducing pathways engaged by the activated Kit. The binding of SCF to Kit induces receptor dimerization, activation of intrinsic tyrosine kinase activity, autophosphorylation and generation of high-affinity binding sites in the kinase insert sequence of Kit that bind signaling molecules and activate distinct signaling cascades [98–111]. According to the current understanding of Kit signaling, a limited number of signaling proteins interact to build multiple interacting networks that allow diverse cellular responses.

Although there is mounting evidence that an activating mutation may enhance Kit signaling and also induce growth factor-independent activation of Kit, there is still limited information about the effect of activating mutations on various aspects of Kit signaling in GISTs, in particular, changes in the activation states of Kit signal transduction pathways that regulate an imbalance that ultimately results in the loss of cell growth and control. Members of the kinase protein family associated with wild-type Kit protein in these systems are summarized in Table 3.

14. Other genetic changes and their pathogenetic role in GISTs

No genes other than c-*kit* have been directly implicated in the pathogenesis of GIST. However, cytogenetic and molecular genetic studies show common losses of chromosomes 14q and 22q in benign and malignant GISTs, suggesting that losses of specific tumor suppressor genes could have a pathogenetic role as early genetic changes [112–118]. Study of neurofibromatosis type 2 (NF2) tumor suppressor gene, mapped to 22q12, infrequently showed mutations in a small series of GISTs, giving no conclusive

Table 3
Summary of the current understanding of the downstream key effector molecules in the Kit signal transduction pathway

Protein (relative molecular mass)	Function in the signal transduction pathway
Src kinase (60 kDa)	Ligand-induced cellular responses such as proliferation, survival, adhesion, and cell migration, as well as ion channel/receptor phosphorylation [98]
Phosphatidylinositol 3′ kinase (PI3K) (85 + 110 kDa)	With its p85 a subunit, generates the second messenger PI (3, 4, 5) P3, a principal (PI3K) activator of cell survival [99]
Tec kinase (72 kDa)	A nonreceptor tyrosine kinase that mediates cellular transformation, and is involved in signals mediated by SCF [100]
Janus-activating kinase 2 (JAK2) (130 kDa)	A nonreceptor tyrosine kinase that is activated via tyrosine phosphorylation in response to cytokines [101,102]
Signal transducers and activators of transcription 5 (STAT5) (59 kDa)	A group of transcription factors induced by JAK kinases in response to cytokine/growth factors IL-2, IL-3, IL-5, GM-CSF, and in some cases, by Src receptors. STATs are thought to play a role in many cancers induced by tyrosine kinases [103–105]
Ras (21 kDa)A guanosine triphosphate/guanosine diphosphate (GTP/GDP) binding protein (G-protein) that activates mitogen-activated protein kinases (MAPK) ERK1 and ERK2.	Ras is activated, either mutationally or by upstream signals in most cancers [106]
Extracellular signal-kinase 1, 2 (ERK1/ERK2) (42/44 kDa)	Proline-directed protein kinases that are activated by dual regulated phosphorylation at threonine and tyrosine residues. They regulate cell cycle, transcription, and differentiation [107]
C-jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK) (46 kDa)	A superfamily member that is activated in response to stress stimuli, growth factors, and cytokines. JNK has a role in regulating cell fate, proliferation, and apoptosis [108,109]
Phospholipase C-γ (PLC) (150 kDa)	Breaks down lipids to form IP-3 and IP-4, which serve as potent second messengers that activate multiple signaling cascades [110,111]

evidence of NF2 gene involvement in GIST pathogenesis [119].

Comparison of benign and malignant GISTs has shown that DNA copy number gains and amplifications in 5p, 8q, 17q and 20q predominantly occur in malignant GISTs, especially in the metastatic tumors. Although no amplified genes have been specifically identified in these regions, these data suggest that the CGH evaluation could have prognostic predictive value [115].

Losses in 9p appear also specific for malignant GISTs [114]. Homozygous loss of cyclin-dependent kinase 4 inhibitor ($P16^{4INK}$) mapped to 9p21 was documented in a small number of malignant GISTs [120]. Losses in the short arm of chromosome 1, especially involving band 1p36, seem to correlate with poor prognosis according to one study [121].

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