Cellular Origin of Gastrointestinal Stromal Tumors

A Study of 27 Cases

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• ***Context.*—Interstitial cells of Cajal (ICCs), also known as pacemaker cells, are cells in the gastrointestinal tract that play a role in the control of gut motility. The ICCs express the c-kit proto-oncogene encoding a type III tyrosine kinase (KIT) receptor, a ligand that is known as stem cell factor (SCF). The maturation of ICCs is dependent on SCF-KIT interaction. The cellular origin, differentiation, nomenclature, and prognosis of gastrointestinal stromal tumors (GISTs) are controversial.**

***Objective.*—To test the hypothesis that GISTs originate from CD34-positive stem cells and differentiate toward an ICC phenotype.**

***Materials and Methods.*—We studied 27 cases of smooth muscle differentiated GISTs collected for 14 years (1985–1999), including 8 benign (leiomyoma), 15 malignant primary (leiomyosarcoma), and 4 metastatic to the liver. Immunohistochemical studies of selected lineage-directed monoclonal antibodies of c-kit (CD117), CD34, vimentin, desmin, α-actin, S100, and MIB-1 were performed on both normal and tumor tissues.**

***Results.*—Immunoperoxidase stains of normal gastrointestinal tract showed both c-kit and CD34-positive cells surrounding the Auerbach ganglia plexus in the gastrointestinal tract. Twenty-seven of 27 tumors strongly expressed c-kit. Fourteen of 27 tumors were positive for CD34. Of the malignant GISTs, 14 of 19 were positive for CD34; of the benign tumors, 0 of 8 were positive for CD34. Thus, CD34 was the best indicator of malignant phenotype.**

***Conclusion.*—This is the first description of benign smooth muscle GISTs negative for CD34. The results of this study suggest that GISTs originate from CD34-positive stem cells and differentiate toward pacemaker cell phenotype. The lack of expression of CD34 in the benign GIST may indicate that benign GISTs are composed of more mature ICCs, whereas malignant GISTs are composed of dedifferentiated ICCs that express CD34-positive stem cells.**

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Gastrointestinal stromal tumors (GISTs) are probably one of the most controversial gastrointestinal tumors in regard to cell origin, differentiation, nomenclature, and prognosis.[1] Historically, Stout[2] assumed that GISTs were derived from smooth muscle based on their morphologic resemblance to smooth muscle tumors in other anatomic sites. Appelman and Helwig have postulated that gastric stromal tumor may originate from mesenchymal cells other than smooth muscle cells.[3–5] The spindle cell neoplasms of the gastrointestinal tract were called *leiomyoma* or *leiomyosarcoma,* and the predominant round or polygonal cell variant was named *epithelioid leiomyoma, leiomyoblastoma,* or *epithelioid leiomyosarcoma.*[3–9] Despite advanced immunohistochemical and ultrastructure studies, the line of differentiation (smooth muscle, neural, gastrointestinal, autonomic nerve, or undifferentiated) is still inconclusive.[10–15] As a result, the noncommittal term *gastrointestinal stromal tumor* has been adopted to describe this heterogeneous group of neoplasms.[16]

Recently, Kindblom et al and others have proposed that most stromal tumors originate from a mesenchymal stem cell that differentiates toward an interstitial cell of Cajal (ICC) phenotype.[17–20] Also known as pacemaker cells, ICCs are a population of cells in the gastrointestinal tract that play a role in the control of gut motility.[21] The ICCs express the c-kit (CD117) proto-oncogene encoding a type III tyrosine kinase (KIT) receptor, a ligand known as stem cell factor (SCF).[22] Maturation of ICC depends on SCF-KIT interaction.[23] Evidence now suggests that CD34-positive stromal tumor, known to be c-kit positive, may differentiate into ICC-like cells.[17,22]

The purpose of the present investigation is to test the hypothesis that GISTs originate from CD34-positive stem cells and differentiate toward an ICC phenotype by immunohistochemical studies using selected lineage-directed monoclonal antibodies for ICCs (c-kit), smooth muscle (desmin), Schwann cells (S100 protein), stem cells (CD34), myofibroblasts (α-actin), and proliferative cells (MIB-1).

MATERIALS AND METHODS

Twenty-seven cases of GISTs were collected for 14 years (1985–1999), including 8 benign (leiomyoma), 15 malignant primary (leiomyosarcoma), and 4 metastatic to the liver. According to Appelman's criterion, we categorized these 27 cases as malignant (>5 mitoses per 50 high-power fields), borderline (<5 mitoses per 50 high-power fields; >5 cm in size), and benign (<5 mitoses

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Table 1. Clinic Data of 27 Gastrointestinal Stromal Tumors

Parameters	Characteristics
Age, y	35–78 (mean, 53; median, 53)
Sex	
Male	12
Female	15
Primary tumors	
Gastric	11
Small bowel	9
Cecum	1
Rectum	1
Abdominal nodule	1
Metastatic tumors to liver	4
Total	**27**

Table 2. Antibody Panel

Antibody	Clone	Source	Dilution
C-kit (CD117)	C-19	Cruz Biotechnology	1:40
CD34	QBEnd/10	Novocastra	1:25
Vimentin	V9	Zymed	1:50
Desmin	ZC18	Zymed	1:50
α-Actin	1A4	Sigma	1:200
S100	. . .	Dako	1:1000
MIB-1	7B11	Zymed	1:200

per 50 high-power fields; <5 cm). The patients ranged in age from 35 to 78 years (mean, 53 years; median, 53 years). The female-male ratio was 15:12. The GISTs were from the stomach (11), small bowel (9), cecum (1), rectum (1), abdominal nodule (1), and metastatic to the liver (4) (Table 1).

Specimens were fixed in 10% buffered formalin, embedded in paraffin tissue, and stained with hematoxylin-eosin using standard procedures. Sections from each case were studied by a modified avidin-biotin complex immunoperoxidase method using a panel of antibodies (Table 2).

RESULTS

The gross features of GISTs usually included a granular, irregular surface with frequent areas of hemorrhage, necrosis, and cystic change (Figure 1). They lacked the characteristic gross appearance of smooth muscle tumors of the uterus in that they did not have a bulging, whorled cut surface. They were commonly well circumscribed, lobulated, or multilobulated and were either partly or predominantly extramural in location. Most tumors were centered in the submucosa, muscularis propria, or both. Both benign and malignant tumors showed ulceration of the overlying mucosa. Therefore, benign and malignant tumors had overlapping gross features and were indistinguishable by gross examination alone except for size.

Microscopically, the benign GISTs (leiomyoma) were composed of spindle cells with pale or eosinophilic fibrillar cytoplasm. The cells varied little in size and shape and were typically arranged in either whorls or long fascicles. Their nuclei were uniform with either low or zero mitotic count. Sometimes, perinuclear vacuoles that indented the nuclei at one pole were identified (Figure 2).

In contrast, malignant or metastatic GISTs (leiomyosarcoma) consisted of high cellularity with an increased nuclear-cytoplasmic ratio. Bizarre cells, nuclear pleomorphism, and mitotic figures were easily found (Figure 3). The cytoplasm was eosinophilic.

Immunoperoxidase stains of normal gastrointestinal tract showed both c-kit and CD34-positive cells surrounding the Auerbach ganglia plexus (Figure 4). Twenty-seven of 27 tumors strongly expressed c-kit (Figure 5). Fourteen of 27 tumors were positive for CD34 (Figure 6). Of the malignant GISTs, 14 of 19 were positive for CD34; of the benign tumors, 0 of 8 were positive for CD34. Thus, CD34 was the best indicator of malignant phenotype (Table 3).

Most GISTs expressed vimentin (25/27) and α-actin (23/27), but desmin and S100 were only positive in 9 and 5 of the 27 tumors, respectively. There was a variable expression of MIB-1 (from 0% to 3% in benign lesions and up to 60% in malignant tumors). All benign GISTs were CD34 negative (0/8), with zero to low expression (<3%) of MIB-1. However, some malignant GISTs (5/19) were CD34 negative, 4 of them from small intestine and 1 liver metastasis (Table 4).

COMMENT

This is the first report that all the benign GISTs stained negative for CD34. The results of this study suggest that GISTs originate from CD34-positive stem cells, which differentiate to express the pacemaker cell phenotype. The lack of expression of CD34 in the benign GISTs may indicate that they are composed of more mature ICCs, whereas malignant GISTs are composed of dedifferentiated ICCs and express stem cell markers (CD34).

The ICCs express KIT receptor. The proto-oncogene c-kit was first identified as the cellular homologue of the oncogene v-kit, found in the feline sarcoma virus, which causes multicentric fibrosarcoma in the domestic cat.[23] The proto-oncogene c-kit is a transmembrane tyrosine kinase receptor that belongs to the same family of receptors as those for platelet-derived growth factor and colony-stimulating factor 1. In human beings, the c-kit gene maps to chromosome 4 (4q11–12) in close proximity to the platelet-derived growth factor receptor and to the gene for epidermal growth factor. The c-kit gene is expressed primarily on hematopoietic stem cells, mast cells, ICCs, melanocytes, and germ cells.

The ultrastructure features of ICCs are many and include subplasmalemmal actin filament bundles, numerous large mitochondria, abundant smooth endoplasmic reticulum, microtubules, caveolae, interdigitating cytoplasmic processes, incomplete external lamina, and synapselike contracts. Thus, these cells have both myoid and neural features, which would explain prior ultrastructural data regarding the electron microscopic features of GISTs.[11]

Vanderwinden and colleagues[24] clearly demonstrated that CD34 and c-kit immunoreactivity reside in closely adjacent, but not overlapping, cell populations in human intestine by double immunofluorescence studies combined with nuclear counterstain and confocal laser microscopy. They found that $CD34^+$ cells are fibroblasts that express the fibroblast marker prolyl 4-hydroxylase. These cells are adjacent to ICCs but distinct from ICCs. These findings challenge the hypothesis that GISTs originate from ICCs.

However, these findings open the possibility that somatic mutations of c-kit may represent part of the oncogenic process rather than an indication of the origin of ICCs per se. This is supported by the finding that CD117, the c-kit proto-oncogene product, is in fact a more specific marker for GISTs than is CD34.[19] In addition, an in-frame

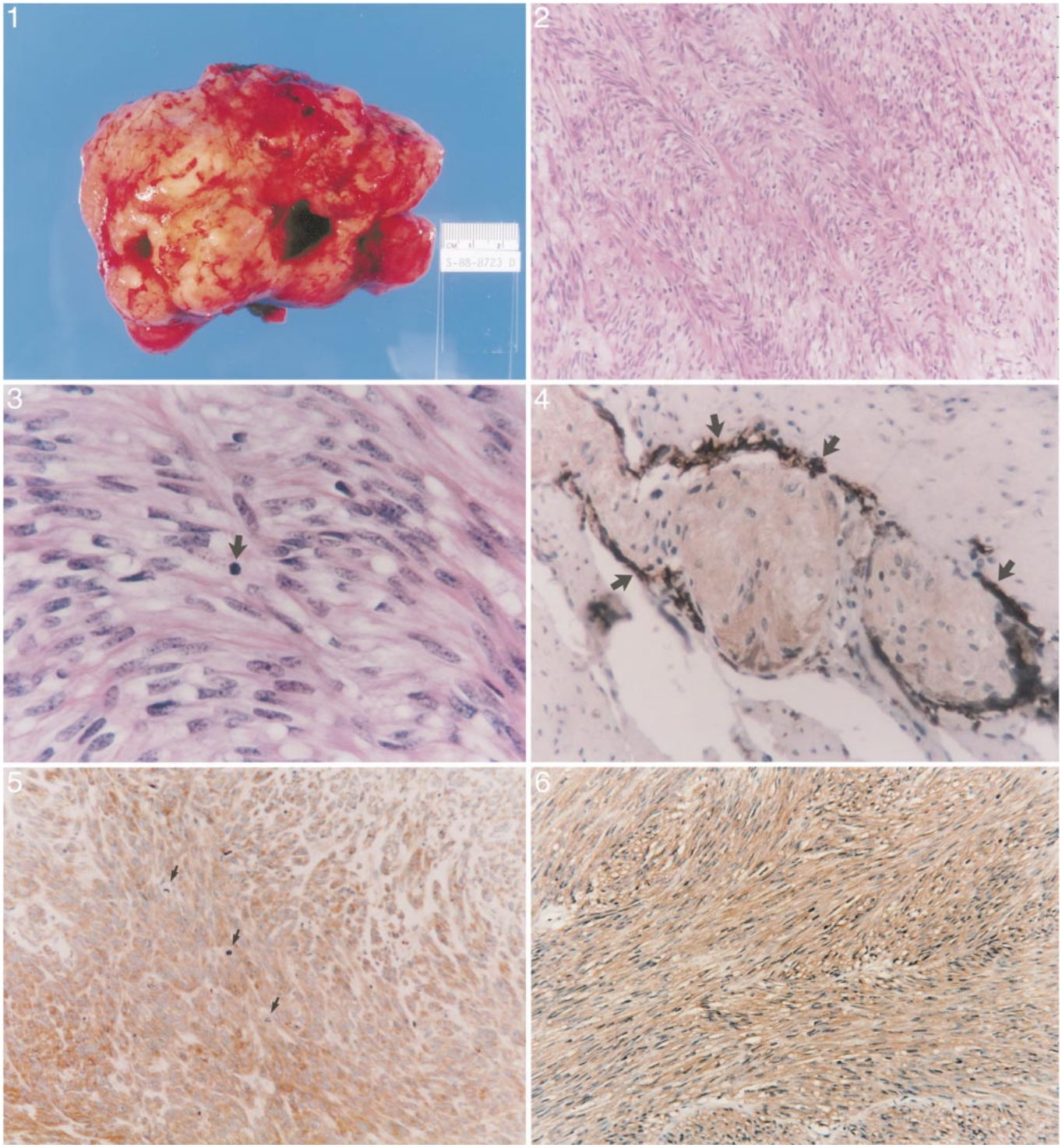

Figure 1. *Grossly, gastrointestinal stromal tumors show irregular surfaces with areas of hemorrhage, necrosis, and cystic change.*

Figure 2. *The spindle cells of the tumor are arranged in long fascicles with perinuclear vacuoles (arrow), which indent the nuclei at one pole (hematoxylin-eosin, original magnification ×156).*

Figure 3. *Hypercellularity, nuclear enlargement, and mitotic figures (arrow) are identified in malignant gastrointestinal stromal tumors (hematoxylin-eosin, original magnification ×624).*

Figure 4. *C-kit (CD117)–positive cells (arrows) surround the Auerbach ganglia plexus in the small intestine (c-kit antibody, original magnification ×312).*

Figure 5. *Gastrointestinal stromal tumors strongly express c-kit (c-kit antibody, original magnification ×312).*

Figure 6. *Some of the gastrointestinal stromal tumors are strongly positive for CD34 (CD34 antibody, original magnification ×156).*

Table 3. Immunohistochemical Analysis of Gastrointestinal Stromal Tumors (GISTs)*

Patient No./Sex/Age, y	Location	Diagnosis	C-kit	CD34	Vimentin	α-Actin	Desmin	S100	MIB-1, %
1/M/43	Stomach	Gastric stromal tumor	+	F+*	+	F+	−	−	3
2/F/51	Stomach	Smooth muscle neoplasm	+	+	F+	F+	−	−	<1
3/M/43	Stomach	Leiomyosarcoma	+	+	+	−	−	−	−
4/M/54	Stomach	Leiomyosarcoma	+	+	+	F+	−	+	1
5/M/52	Stomach	Leiomyosarcoma	+	+	+	−	+	−	−
6/F/62	Stomach	Gastric stromal tumor	+	+	+	+	−	−	<1
7/F/53	Stomach	GIST	+	+	+	F+	F+	−	3
8/M/50	Stomach	Leiomyosarcoma	+	+	+	−	−	−	<1
9/F/78	Small intestine	GIST, Smooth muscle differentiation	F+	−	−	+	+	−	<1
10/M/44	Small intestine	Leiomyosarcoma	+	−	+	+	+	−	−
11/F/49	Small intestine	Malignant stromal tumor from leiomyosarcoma	+	−	F+	+	−	−	−
12/M/60	Small intestine	Malignant stromal tumor	+	−	+	−	−	−	<1
13/F/63	Small intestine	Neurofibromatosis	+	+	+	F+	−	−	<1
14/M/63	Duodena	Malignant stromal tumor from leiomyosarcoma	+	F+	+	F+	−	+	5
15/M/64	Rectum	Malignant stromal tumor	F+	+	+	F+	−	−	60
16/F/58	Liver	Leiomyosarcoma (metastatic)	+	+	+	+	−	−	−
17/F/56	Liver	Leiomyosarcoma (metastatic)	+	+	+	+	−	−	40
18/F/53	Liver	Leiomyosarcoma (metastatic)	+	−	+	+	+	−	50
19/M/46	Liver	Leiomyosarcoma (metastatic)	+	+	+	+	−	−	−
20/M/58	Gastrointestinal junction	Leiomyoma	+	−	+	+	+	+	−
21/M/55	Gastrointestinal junction	Leiomyoma	+	−	+	+	+	−	−
22/F/49	Abdominal mass	Leiomyoma	+	−	+	+	F+	−	<1
23/F/46	Small intestine	Leiomyoma	+	−	+	+	+	−	−
24/F/37	Small intestine	Stromal tumor	+	−	−	+	−	+	−
25/F/35	Cecum	GIST	+	−	+	F+	−	F+	<1
26/F/54	Stomach	GIST	+	−	+	+	−	−	3
27/F/61	Small intestine	GIST	+	−	+	+	−	−	−

* F indicates focal; plus sign, positive; and minus sign, negative.

Table 4. Immunohistochemical Analysis of 27 Gastrointestinal Stromal Tumors (GISTs)

GISTs	C-kit	CD34	Vimentin	α-Actin	Desmin	S100	MIB-1, %
Benign (8)	8/8	0/8	7/8	8/8	4/8	3/8	0–3
Malignant (19)							
Stomach (8)	8/8	8/8	8/8	5/8	2/8	1/8	0–3
Small intestine (5)	5/5	0/5	4/5	4/5	2/5	1/5	0–<1
Duodenum (1)	1/1	1/1	1/1	1/1	0/1	0/1	5
Rectum (1)	1/1	1/1	1/1	1/1	0/1	0/1	60
Metastatic to liver (4)	4/4	3/4	4/4	3/4	1/3	0/3	0–50

deletion or a point mutation in exon 11 of c-kit occurs preferentially in malignant versus benign GISTs,[25] and c-kit mutation is associated with a poor prognosis in patients with GISTs.[26] There is also a report of familial GISTs with a germline mutation (exon 11 deletion between the transmembrane and tyrosine kinase domain) of the c-kit gene.[27] A report of a woman with leiomyomatosis and a leiomyosarcoma arising from a leiomyoma may indicate c-kit is important in the development and malignant transformation of GISTs.[28] All these may explain why malignant GISTs express both c-kit and CD34 but benign GISTs express c-kit only. This is possibly attributable to gain-of-function mutations of the c-kit gene.[22]

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