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Gastrointestinal Pacemaker Cell Tumor (GIPACT)

Gastrointestinal Stromal Tumors Show Phenotypic Characteristics of the Interstitial Cells of Cajal

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The interstitial cells of Cajal (ICC) form a complex cell network within the gastrointestinal tract wall where they function as a pacemaker system. Expression of the kit proto-oncogene is essential for the development of this system. The aim of our study was to examine the hypothesis that gastrointestinal stromal tumors differentiate toward cells with an ICC phenotype. Ultrastructurally, 58 stromal tumors were characterized and found to share many features with ICC. Seventy-eight stromal tumors were immunophenotyped, particularly with regard to the kit receptor. All 78 tumors revealed strong, homogeneous immunoreactivity for the kit receptor as did ICC of adjacent and control gastrointestinal walls. Focal hyperplasia and hypertrophy of kit receptor positive cells were also observed in the gastrointestinal wall adjacent to the tumors. CD34 immunoreactivity observed in interstitial cells surrounding Auerbach's ganglia suggests that a subpopulation of ICC is CD34 positive and may explain why 56 of 78 stromal tumors were CD34 positive. Thirty control tumors, including gastrointestinal leiomyomas and leiomyosarcomas, were all negative for the kit receptor. We conclude that gastrointestinal stromal tumors show striking morphological and immunophenotypic similarities with ICC and that they may originate from stem cells that differentiate toward a pacemaker cell phenotype. We propose that the noncommittal name "gastrointestinal stromal tumor" be replaced by gastrointestinal pacemaker cell tumor. *(Am J Pathol 1998, 152:1259–1269)*

Despite numerous studies, gastrointestinal stromal tumors remain problematic with regard to origin, differentiation, nomenclature, and prediction of prognosis. Their morphological spectrum is wide, ranging from bland to frankly malignant tumors with spindled and/or epithelioid appearances.[1–3] Hence, a variety of names such as epithelioid or bizarre leiomyomas, epithelioid leiomyosarcomas or leiomyoblastomas, and gastrointestinal autonomic nerve tumors (GANT) have been used for these tumors reflecting the various views regarding their differentiation, classification, and prognosis.[1–8] The noncommittal term gastrointestinal stromal tumor has recently gained wide acceptance, emphasizing their enigmatic origin and the fact that most of these lesions do not display convincing smooth muscle or neuronal differentiation.[2,9,10]

The existence of a complex system of interstitial cells of Cajal (ICC), which are intercalated between the autonomic nerves and the muscle walls of the gastrointestinal tract, has been known for over 100 years.[11] Detailed morphological and electrophysiological studies in many species, including humans, have indicated that ICC have a pacemaker function.[11–18] Recently, ICC were found to express the kit proto-oncogene, which encodes for a transmembrane tyrosine-kinase receptor (CD117) and has the stem cell factor as its ligand. Expression of the kit gene is essential for the development of normal hematopoiesis, proliferation, and migration of primordial germ cells and melanoblasts during embryogenesis as well as for the development of the ICC and gastrointestinal pacemaker activity.[19–28] A cluster of human type III receptor protein tyrosine kinase genes, including the kit gene, has been mapped to chromosome 4q11-q12.[29]

The present study was designed to test the hypothesis that gastrointestinal stromal tumors differentiate toward an ICC phenotype. Ultrastructural examination and immunohistochemical analysis for the kit tyrosine-kinase receptor (CD117) was performed in a large series of well-characterized stromal tumors along with appropriate normal tissues and tumor controls. The results of this study support our hypothesis that gastrointestinal stromal tumors originate from a stem cell that differentiates toward an ICC phenotype.

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Table 1. Antibodies, Dilutions, and Sources

Antibody	Clone	Pretreatment	Antibody dilution	TechMate protocol	Source
c-kit (C-19)	Poly	mw	1:40	MSIP	1
c-kit (K089)	Poly	mw	1:25	MSIP	2
CD 34	QBend10	mw	1:50	MSIP	3
von Willebrand factor	F8/86	mw	1:50	MSIP	4
CD 31	JC/70A	mw	1:80	MSIPE	4
Vimentin	V9	mw	1:400	MSIP	4
Desmin	33	mw	1:400	MSIP	4
α-SMA	1A4	None	1:200	MSIP	4
Muscle specific actin	HHF 35	None	1:200	MSIP	4
S-100 protein	Poly	None	1:4000	MSIP	4
Neurofilament	2F11	mw	1:600	MSIP	4
Chromogranin	DAK-A3	mw	1:1000	MSIP	4
PGP 9.5	Poly	mw	1:2000	MSIP	5

Abbreviations: mw, microwave treatment in citrate buffer (Dako ChemMate™ buffer for antigen retrieval).
All stainings were performed in a Dako TechMate™ immunostainer. Using the ChemMate™ Detection Kit, the MSIP protocol amplifies the primary antibody reaction by a sequential application of biotinylated antibodies to rabbit/mouse immunoglobulins followed by peroxidase-conjugated streptavidin. The enzyme is visualized using a chromogenic peroxidase substrate solution. The MSIPE protocol includes an initial step with proteinase K digestion.
Source: 1, Santa Cruz Biotechnology, Santa Cruz, CA; 2, Immuno-Biological Laboratories, Tokyo, Japan; 3, Bionotics, Wyboston, Bedfordshire, UK; 4, Dakopatts, Glostrup, Denmark; 5, Ultra Clone, Wellow, Isle of Wight, UK.

Materials and Methods

Gastrointestinal Stromal Tumors (GIST), Control Tissues, and Tumors

A total of 78 cases of GIST were retrieved from the files of the Department of Pathology, Sahlgrenska University Hospital, Gothenburg, Sweden. The stromal tumors were chosen to include histologically benign, borderline, and overtly malignant primary tumors (65) as well as metastases (13) to peritoneal surfaces, lymph nodes, and liver. Control tissues included segments of normal human gastric, small intestinal, and colonic walls. Gastrointestinal walls at resection margins and adjacent to the tumors served as internal controls. Thirty control tumors were also analyzed, including leiomyoma (four gastrointestinal, three cutaneous, and three uterine), leiomyosarcoma (two gastrointestinal, four soft tissue, and two uterine), schwannoma (two gastrointestinal and one soft tissue), carcinoid (four gastrointestinal), malignant fibrous histiocytoma (two gastrointestinal), inflammatory fibrosarcoma (one gastrointestinal), angiosarcoma (one gastrointestinal), and one metastatic melanoma to the gastrointestinal tract.

Immunohistochemical and Ultrastructural Studies

The immunohistochemical techniques, antibodies, dilutions, and sources are summarized in Table 1. All antibodies were applied to 78 stromal tumors and 30 control tumors. Serial cut sections from control gastrointestinal walls and selected stromal tumors were used to correlate the distribution of immunoreactivity for kit receptor, CD34, PGP 9.5, and endothelial markers. Fifty-eight stromal tumors were analyzed ultrastructurally; 38 cases were primarily fixed in 4% glutaraldehyde, and 20 cases were formalin-fixed and processed for ultrastructural analysis. The preservation of the 20 formalin-fixed cases was somewhat variable but in all cases was adequate for evaluation of the recorded morphological features.

Results

Clinical Data

The clinical data are summarized and tabulated in Table 2.

Light Microscopy

Of the 65 primary stromal tumors, 28 were predominantly epithelioid, 20 predominantly spindled, and 17 were an admixture of epithelioid and spindled cells (Figure 1, a to c). Twelve primary stromal tumors were classified as histologically benign based on their low mitotic activity (<5 mitoses/50 hpf), bland cytological features, and absence of necrosis and mucosal infil-

Table 2. Summary of Clinical Data in 78 GIPACT

Parameters	Characteristics
Age	15 to 87 years (median, 57 years)
Sex distribution	
Males	39
Females	39
Tumor site	
Primary tumors	65 (total)
Gastric	42
Small intestine	16
Colon-rectum	7
Metastatic Tumors	13 (total)
Liver	7
Lymph nodes	1
Peritoneum	5
Tumor Size	
Primary tumors	2 to 25 cm (median, 8 cm)
Metastatic tumors	1 to 17 cm (median, 4 cm)

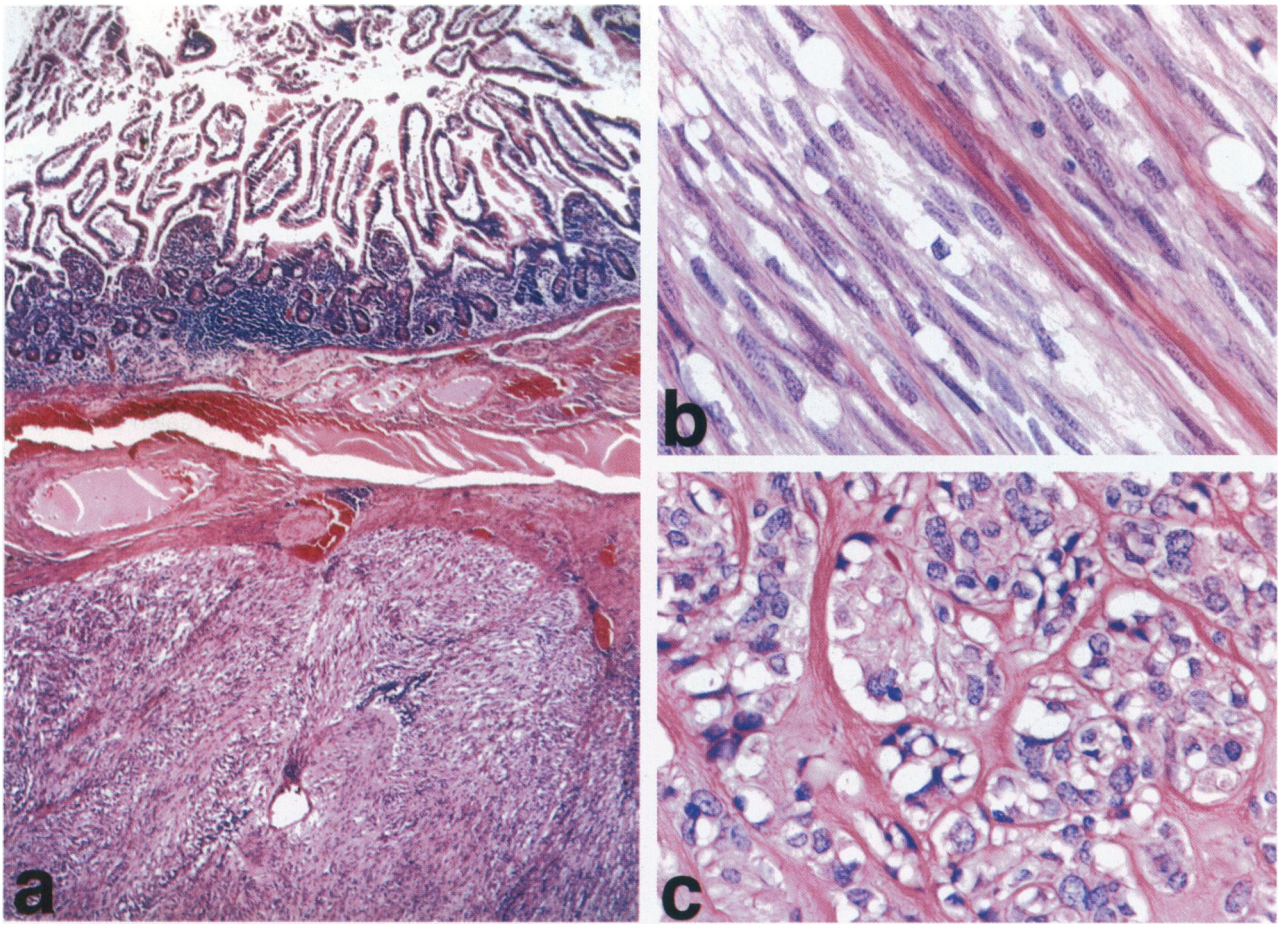

Figure 1. Gastrointestinal stromal tumor involving the muscularis propria of the ileum (**a**). The tumor displays both spindled (**b**) and epithelioid (**c**) features. Note the characteristic vacuoles of the tumor cell cytoplasm. Magnification, ×30 (**a**), ×300 (**b** and **c**).

tration. The remaining 53 cases were either frankly malignant with high mitotic activity (≥5 mitoses/50 hpf), prominent cellular and nuclear atypia, areas of necrosis and hemorrhage, and frequent mucosal infiltration, or they possessed features intermediate or borderline between the benign and overtly malignant tumors and were therefore considered to be of indeterminate or uncertain biological potential. Of the 13 metastatic stromal tumors, nine were predominantly epithelioid and four were predominantly spindled.

Ultrastructural Appearance — GIST Resemble Interstitial Cells of Cajal

The 58 stromal tumors (47 primary and 11 metastatic) displayed a wide spectrum of cellular differentiation both within and between cases, clearly deviating in appearance from adjacent smooth muscle. A striking feature seen at least focally in both the spindled and epithelioid tumor cells of 40 cases was the presence of prominent delicate filopodia-like cytoplasmic projections that interdigitated in a complex fashion (Figure 2) and sometimes invaginated deeply into neighboring cells. The interdigitating cell processes were usually closely apposed without any specialized contacts, but in some areas there were numerous desmosome-like (adhering-type) junctions (Figure 3) as well as a few gap junctions. The most striking cytoplasmic features included an abundance of large mitochondria (Figure 4a), prominent tubular cisternae of smooth endoplasmic reticulum (Figure 5) and large Golgi zones (Figure 4, a and b), networks of intermediate filaments (Figure 4a), and microtubules. In 10 cases, there were scattered tumor cells with bundles of thin filaments of actin type, occasionally forming elongated condensations (Figure 6). Surface caveolae were irregularly distributed along the cytoplasmic membrane. The tumor cells were enclosed by an incomplete external lamina or lacked external lamina entirely. There were scattered or grouped dense core granules, mostly 80 to 200 nm in diameter within cytoplasmic processes or more commonly associated with the Golgi zones (Figure 4b). In six cases, the tumor cells were closely associated with rare bulbous, synapse-like structures containing clusters of dense core granules (Figure 7) or small empty vesicles. The intercellular matrix included a sparse amount of collagen and occasionally fine, granular, fibrillar material resembling elastin. There were also aggregates of so-called skeinoid fibers in the matrix of three tumors.

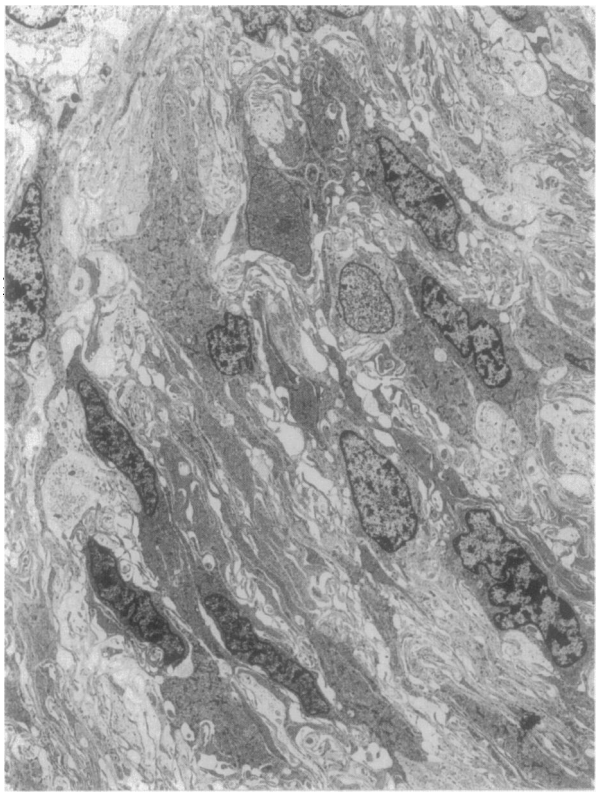

Figure 2. Spindle-shaped tumor cells with elongated, clefted nuclei and elaborate, complex systems of long, delicate interdigitating cytoplasmic processes. Some cytoplasmic projections invaginate deeply into the cytoplasmic recesses of neighboring cells. Magnification, ×2300.

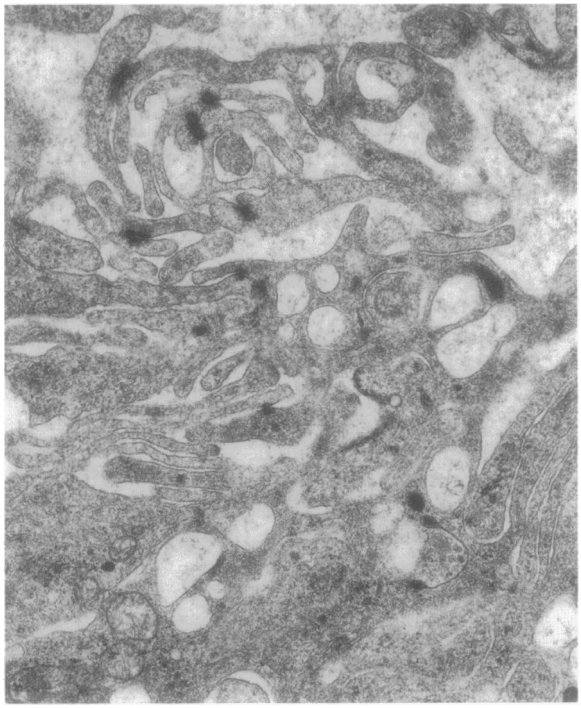

Figure 3. The cytoplasmic processes are focally connected by adhering-type, desmosome-like junctions. Magnification, ×16,000.

Immunohistochemistry

The Pacemaker Cell System

Immunostaining for the kit receptor in gastrointestinal control tissues unveiled the ICC as an intricate system of fusiform cells with thin, elongated bipolar or dendritic-like cytoplasmic projections throughout the gastrointestinal tract. Most ICC were located between the circular and longitudinal layers of the muscularis propria (Figure 8a), intimately associated with or enclosing Auerbach's ganglia (Figure 8b), and sprouting out into the smooth muscle walls (Figure 8c). Both kit receptor antibodies (C-19 and K089) gave identical results. With the exception of mast cells, no other cells or tissue revealed any kit positivity. In the gastrointestinal wall of six cases, there were foci with increased numbers of kit positive cells that were near the tumors; the kit positive cells were diffusely distributed within the gastrointestinal muscle wall in these areas (Figure 8, d and e). Typically these cells were spindled or stellate and somewhat enlarged compared with corresponding kit positive cells in the nontumor controls, having more abundant cytoplasm and hyperchromatic, large nuclei with prominent nucleoli (Figure 8f; compare with Figure 8c).

The immunoreactions for CD34 performed on consecutive sections of the same control tissues revealed distinctly positive cells intimately associated with the ganglia of Auerbach's plexus (Figure 9a). There were also scattered CD34 positive cells running between the circular and longitudinal muscle layers of the gastrointestinal wall as well as a few sprouting cells within the smooth muscle walls. The CD34 immunostaining, however, identified only a small fraction of the cells identified with the kit-receptor antibodies. In the five cases with focal diffuse hyperplasia of kit positive cells adjacent to GIST, the CD34 immunostaining produced a similar result with a striking increase in the number of CD34 positive cells that intermingled with the smooth muscle of the gastrointestinal wall (Figure 9b; compare Figure 8d, which is a consecutive section of the same case). Because CD34 positivity is also observed in endothelial cells, consecutive sections of the control tissues were also analyzed for the endothelial markers, CD31 and von Willebrand factor. The spindle cells corresponding to the CD34 positive cells observed in the walls of gastrointestinal controls and adjacent to stromal tumors were all negative for these endothelial markers. Biopsies of normal skin were also analyzed for CD34 and kit receptor immunoreactivity. In addition to endothelial cells, a population of dendritic dermal stromal cells were CD34 positive. In consecutive sections immunostained for kit receptor, normal melanocytes of the epidermis were positive; no perivascular or periadnexal cell population showed kit receptor positivity.

The PGP 9.5 antibody produced distinct and strong immunostaining of nerve cells throughout the gastrointestinal walls but did not identify the kit positive ICC.

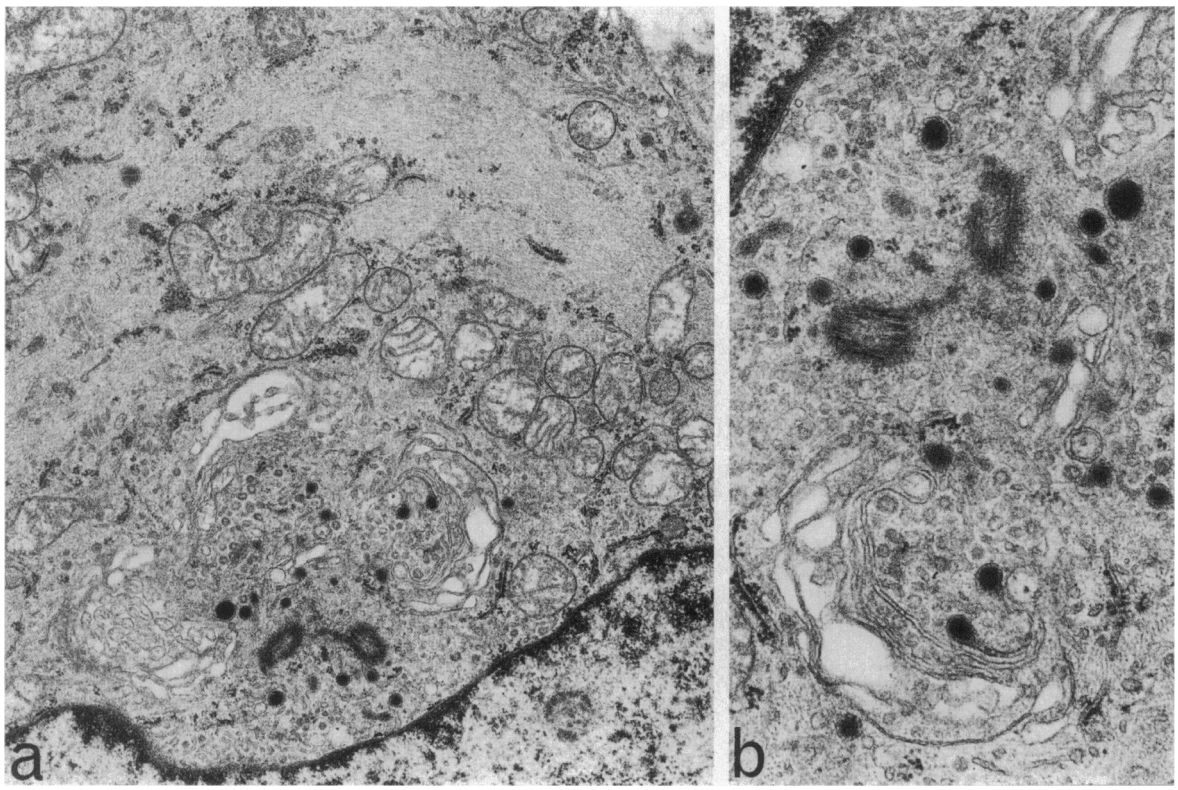

Figure 4. Tumor cell with a prominent perinuclear Golgi zone, numerous mitochondria, and abundant intermediate filaments. Associated with the Golgi zone are a pair of centrioles and small dense core granules. Magnification, ×15,000 (**a**), ×30,000 (**b**).

GIST

The immunohistochemical results are summarized in Tables 3 and 4. All 78 stromal tumors showed strong immunoreactivity with the C-19 kit receptor antibody and all but one with the K089 kit antibody. The cytoplasmic staining was typically granular and homogenous throughout the tumors (Figure 8, g to i). The surrounding smooth muscle of the gastrointestinal wall was negative (Figure 8g). The distribution and intensity of the immunoreaction for the kit receptor were not related to histological pattern, gastrointestinal location, malignant potential of the tumor, or whether the lesion was primary or metastatic.

The CD34 immunoreactions performed on consecutive sections revealed strong and diffuse positivity (Figure 9c) similar to that of the kit-receptor staining in 56 of 78 stromal tumors (49 of 65 primary tumors and 7 of 13 metastatic tumors). The remaining 22 tumors were entirely negative. CD34 immunostaining was unrelated to histological type, location, and classification as benign, borderline, or malignant.

Control Tumors

All 30 control tumors were negative for the kit receptor. Twenty-seven of the control tumors were negative for CD34, whereas focal positivity was seen in two small intestinal schwannomas and an intestinal epithelioid angiosarcoma. Notably, all of the gastrointestinal leiomyomas[4] and rectal leiomyosarcomas[2] showed light micro-

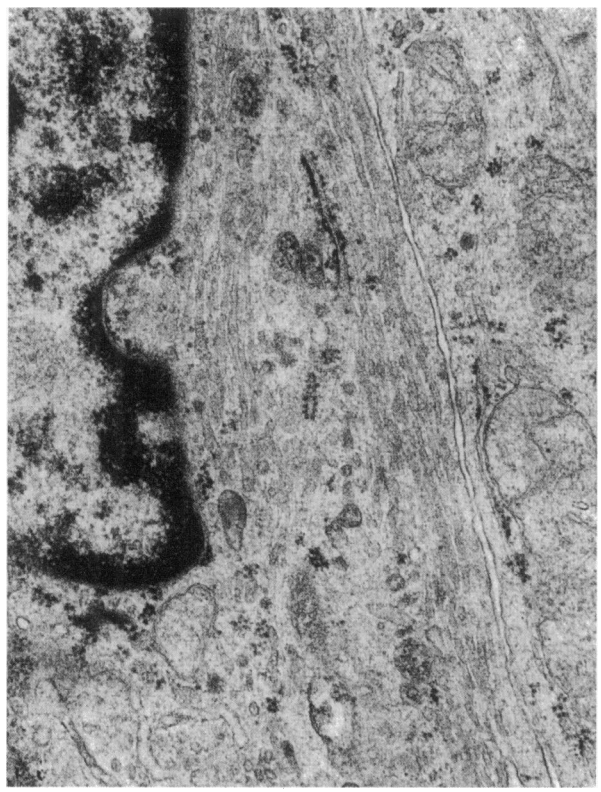

Figure 5. Detail of two closely apposed tumor cells, one of which shows an abundance of smooth endoplasmic reticulum arranged in parallel tubular cisternae. Magnification, ×30,000.

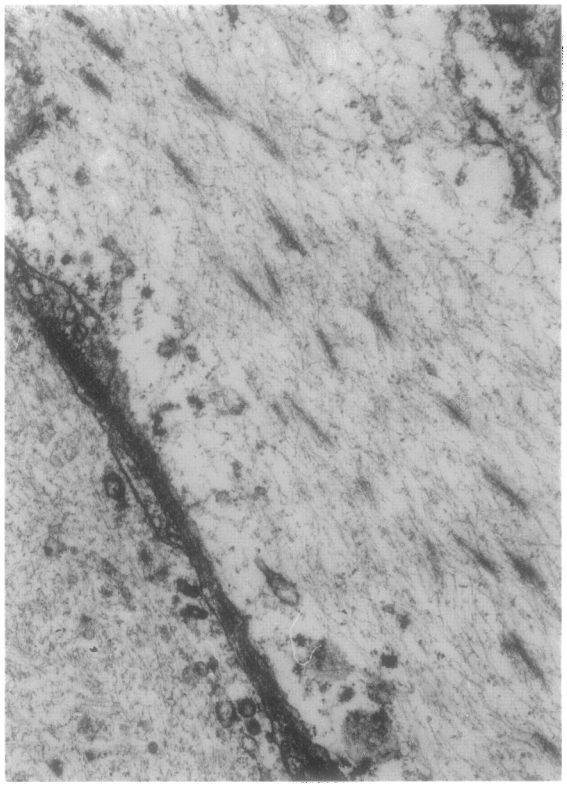

Figure 6. Detail of two tumor cells from a metastatic tumor in the liver. One of the cells contains an abundance of intermediate filaments and thin filaments of actin-type with longitudinal condensations. Magnification, ×18,000.

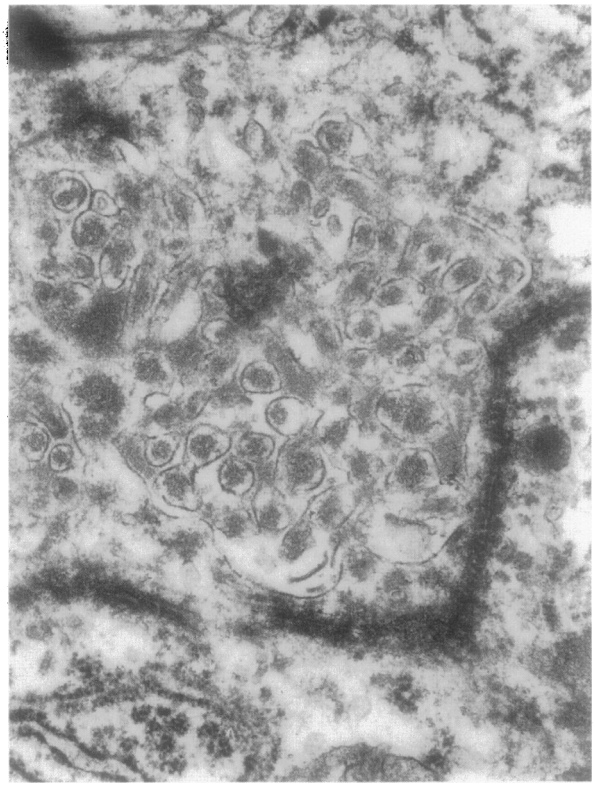

Figure 7. Tumor cell intimately associated with a bulbous, synapse-like structure containing numerous dense core granules. Magnification, ×30,000.

scopic features indicating smooth muscle differentiation and strong immunoreactivity for desmin, α-smooth muscle actin, and muscle specific actin (HHF 35). PGP 9.5 immunoreactivity was observed in two leiomyomas, two schwannomas, two malignant fibrous histiocytomas, and all four carcinoids.

Discussion

Gastrointestinal stromal tumor is the most frequent nonepithelial tumor occurring in the stomach and small bowel.[1,2,4,30] The confusion and controversy surrounding this tumor are related to its enigmatic origin and difficulty in identifying reliable prognostic criteria. In the past, they were considered to be smooth muscle in origin,[1–4,31] but recent studies have shown a more complex picture with evidence of smooth muscle differentiation, neural differentiation (referred to as GANT, myenteric plexus tumors, or plexosarcomas), dual smooth muscle and neuronal differentiation, or no differentiation (undifferentiated mesenchyme).[32–34] The clinical, morphological, immunophenotypic, and ultrastructural overlap of these tumors suggest these tumors are an entity, hence the unifying but noncommittal term gastrointestinal stromal tumor was introduced.[1,2,4,10] Although the term refers to the mesenchymal nature of the lesion, the term gastrointestinal stromal tumor, in general, does not include tumors of the gastrointestinal tract with true smooth muscle, fibroblastic, schwannian, or vascular differentiation. In accordance with this definition of gastrointestinal stromal tumor, the 78 tumors of this series displayed relatively uniform ultrastructural and immunophenotypic characteristics. Thus, none of the tumors revealed definite smooth muscle differentiation. In contrast to normal smooth muscle and *bona fide* benign and malignant smooth muscle tumors of the gastrointestinal tract and other sites that were analyzed for comparison, the stromal tumors of our series were all immunonegative for desmin, muscle specific actin (HHF 35), and in the majority of cases for smooth muscle actin. The ultrastructural characteristics also deviated from true smooth muscle tumors of the gastrointestinal tract and other sites. They uniformly lacked thick filaments of myosin type. There was, however, a small group of stromal tumors that expressed smooth muscle actin and ultrastructurally displayed bundles of actin-type filaments with elongated condensations. This finding has previously been interpreted as true smooth muscle differentation.[32] An alternative interpretation is that the incomplete myoid differentiation seen in stromal tumors reflects the myoid features of ICC.[11,18] ICC may, in fact, be derived from smooth muscle progenitor cells.[11,18]

Other than occasional tumor cells containing dense core granules and rarely associated with synapse-like structures, the tumors revealed no ultrastructural features of neuronal differentiation. Moreover, the tumors were uniformly negative for S-100 protein, neurofilaments, and chromogranin. The strong PGP 9.5 immunoreactivity observed in the majority of our cases is of interest in view of

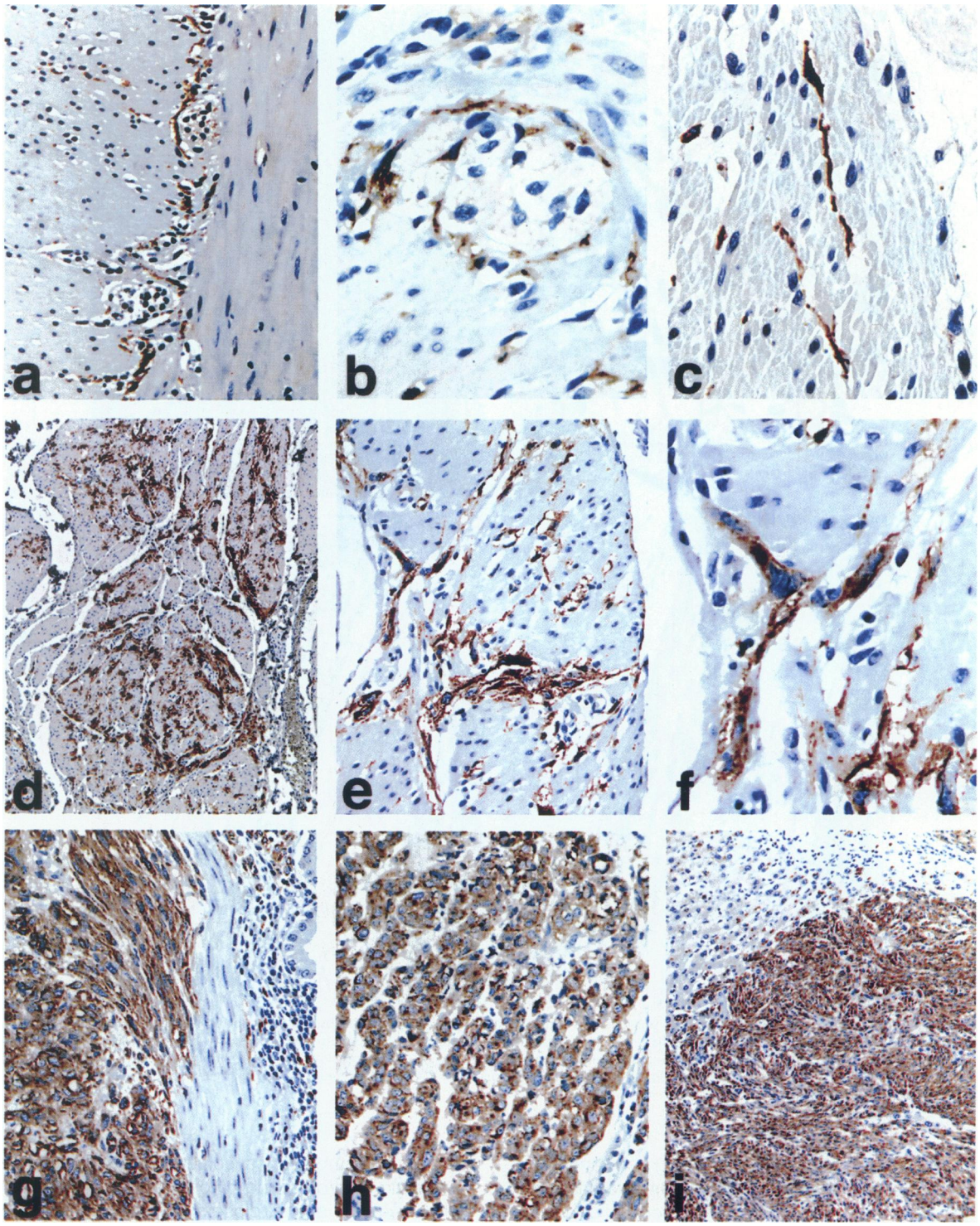

Figure 8. Immunoreactivity for the kit-receptor identifies the pacemaker (ICC) cells running between the circular and longitudinal muscle layers of the gastrointestinal muscle wall (**a**), encircling the Auerbach's autonomic ganglia (**b**), and forming long, delicate bipolar cytoplasmic projections in intimate contact with the smooth muscle cells (**c**). At a distance from the tumors there are areas with prominent hyperplasia of the kit-positive cells (**d**). These cells appear hypertrophic with nuclear enlargement and hyperchromasia (**e** and **f**). Strong kit-receptor immunoreactivity in a spindled tumor of the gastric antrum (**g**), an epithelioid tumor of the ileum (**h**), and a metastasis to the liver (i). Magnification, ×75 (**a, g, h,** and i), ×300 (**b, c,** and **f**), ×50 (**d**), ×150 (**e**).

the fact that its detection in some stromal tumors and GANT has been interpreted as evidence of neural differentiation.[33,34] We found PGP 9.5, an antibody directed against a soluble protein of unknown function originally isolated from brain,[35] to be an excellent nerve cell marker in the gastrointestinal tract. However, its specificity as a neural marker in tumors is questionable. Moderate to strong PGP 9.5 immunoreactivity was observed in 10 of

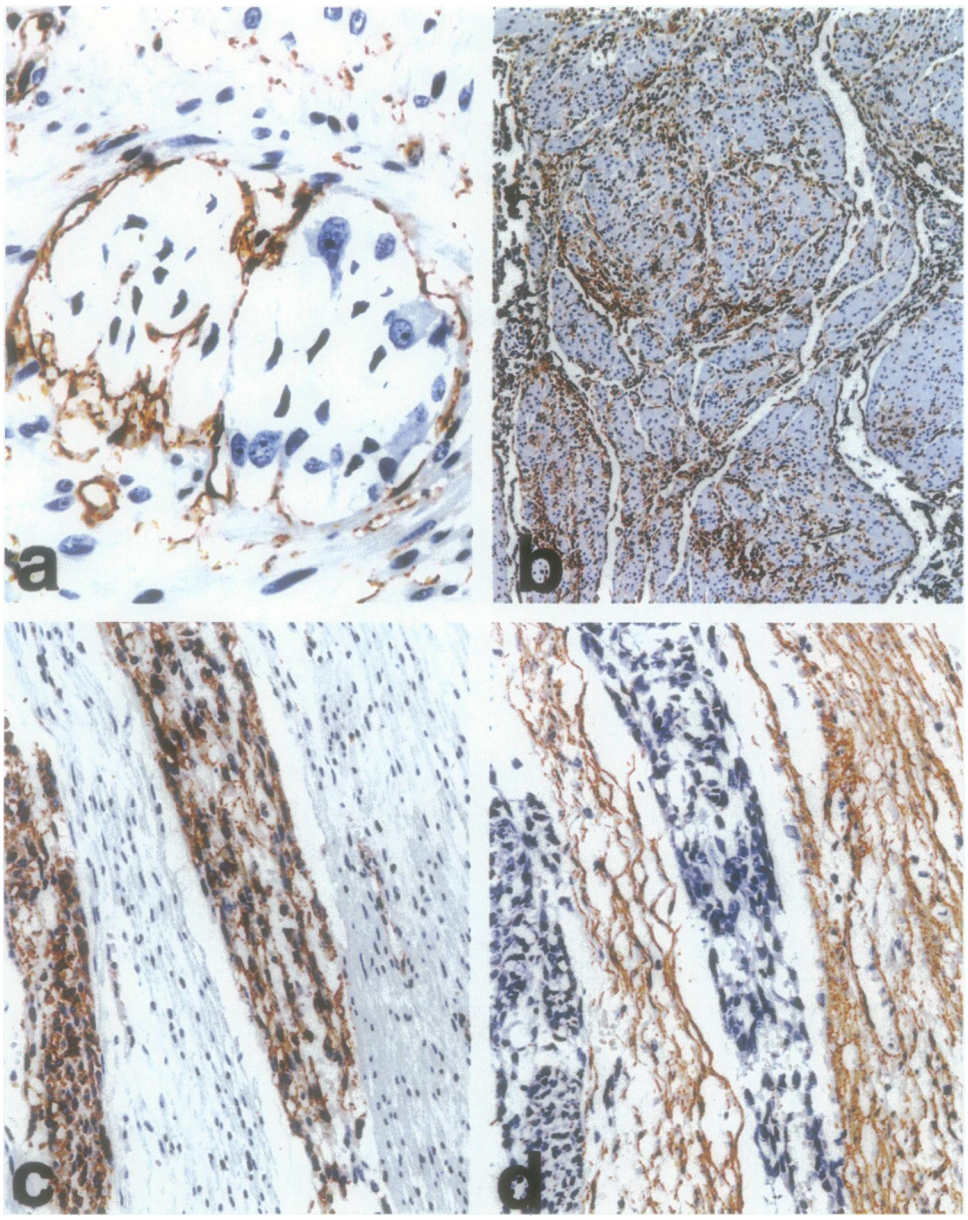

Figure 9. a: Auerbach's ganglion surrounded by CD34 positive cells with a similar appearance and distribution to the kit positive pacemaker cells. **b:** At a distance from the tumor, focal proliferation of CD34 positive cells is noted. Compare Figures 8e and 9b; consecutive 4-micron sections have been stained for the kit-receptor and CD34, respectively. **c:** GIST/GIPACT infiltrating the gastrointestinal smooth muscle wall; the tumor shows strong CD34 positivity in contrast to the surrounding muscle. **d:** Same area as in **c**; the smooth muscle bundles show strong immunoreactivity for α-smooth muscle actin, whereas the tumor is negative. Magnification, ×300 (**a**), ×50 (**b**), ×100 (**c** and **d**).

30 control tumors in our study, including schwannomas, leiomyomas, and malignant fibrous histiocytomas. PGP 9.5 has also been observed by others in classical leiomyomas and schwannomas.[36]

The ultrastructural findings in this large series of stromal tumors, which included benign to overtly malignant primary and metastatic tumors with spindled to epithelioid features, showed striking similarities to ICC. Thus, the spectrum of ultrastructural features observed in this study, including incomplete myoid differentiation with subplasmalemmal actin filament bundles and occasional condensations, numerous large mitochondria, abundance of smooth endoplasmic reticulum, prominent Golgi zones, microtubules, caveolae, networks of intermediate filaments, interdigitating cytoplasmic processes, incomplete external lamina, close apposition of cells with desmosome-like and rare gap-junctions, and synapse-like contacts, are all features that are typical of ICC.[11–12,15–18]

The identification of GANT as a subgroup of stromal tumors rests mainly on ultrastructural identification of tumor cells with prominent axon-like cytoplasmic processes, loosely organized intermediate filaments, scattered microtubules and occasional dense-core granules and tumor cells associated with bulbous synapse-like structures.[5–7,37–39] Many tumors in our series displayed these features as well as other characteristics of gastro-

Table 3. Immunohistochemical Analysis of Kit Receptor and CD34 in 78 GIPACT and 30 Control Tumors

Material	Antibody and number + cases/total cases		
	c-19 (kit receptor)	K089 (kit receptor)	CD34
GIPACT			
Primary	65/65	64/65	49/65
Metastatic	13/13	13/13	7/13
Control tumors			
Gastrointestinal tract			
Leiomyoma*	0/4	0/4	0/4
Leiomyosarcoma*	0/2	0/2	0/2
Schwannoma	0/3	0/3	2/3
Angiosarcoma	0/1	0/1	1/1
Malignant fibrous histiocytoma	0/2	0/2	0/2
Inflammatory fibrosarcoma	0/1	0/1	0/1
Metastatic melanoma	0/1	0/1	0/1
Carcinoid	0/4	0/4	0/4
Other sites			
Leiomyoma (skin and uterus)*	0/6	0/6	0/6
Leiomyosarcoma (uterus and soft tissue)*	0/6	0/6	0/6

*All 18 leiomyomas and leiomyosarcomas were strongly immunoreactive for desmin, muscle specific actin, and α-smooth muscle actin.

intestinal pacemaker cells. However, the clinical, histological, and immunophenotypic features of the six tumors of our series with GANT-like features (synapse-like structures) and cases previously reported as GANT did not differ from the other stromal tumors in our series. The ultrastructural features described as being diagnostic of GANT are, in fact, characteristics of gastrointestinal pacemaker cells. It is therefore possible that tumors previously described as GANT are part of a neoplastic spectrum with GANT displaying a higher degree of ICC differentiation, including neuronal contacts, than is seen in most stromal tumors.

The recent discovery of the selective expression of the kit receptor in ICC within the gastrointestinal tract and its crucial role in the normal development of the ICC system and pacemaker activity[19,21–23] has produced a powerful new tool in the investigation of these cells and their function in normal and pathological conditions. A lack of pacemaker cells has been observed in aganglionic segments of the colon in Hirschsprung's disease.[40,41] These findings and our own ultrastructural observations in stromal tumors prompted us to explore the hypothesis that stromal tumors differentiate toward an ICC phenotype. The striking, uniform immunoreactivity for kit receptor found in our stromal tumors and its absence in the control tumors corroborate our ultrastructural findings. Kit receptor positivity in the stromal tumors was unrelated to morphological (spindled or epithelioid), immunohistochemical (CD34 or PGP 9.5 positive), and ultrastructural features (myoid features or GANT-like) as well as malignancy grade, suggesting that stromal tumors represent a single entity with a wide morphological spectrum and various degrees of differentiation toward an ICC phenotype. The selective expression of the kit receptor in ICC of the gastrointestinal tract along with the high degree of ICC differentiation seen ultrastructurally in stromal tumors indicate that the kit receptor positivity observed in stromal tumors is a reflection of ICC differentiation rather than expression of nonspecific stem cell features. We propose the name gastrointestinal pacemaker cell tumor (GIPACT) as a unifying concept and as a replacement for the noncommittal term gastrointestinal stromal tumor as it has been defined (lacking obvious smooth muscle, schwannian, or other differentiating features).[42]

Table 4. Immunohistochemical Analysis of 78 GIPACT

Antibody	Positive cases/total number of cases
Vimentin	78/78
PGP 9.5	55/78
α-smooth muscle actin	12/78
Desmin	0/78
Muscle specific actin	0/78
S-100 protein	0/78
Neurofilament	0/78
Chromogranin	0/78

The results of our study also indicate that the kit receptor is a useful diagnostic marker for stromal tumors/GIPACT that have a wide differential diagnosis in view of their variable histological appearances and the fact that metastases may precede detection of the primary tumor. All 30 control tumors, which included true smooth muscle and schwannian tumors of the gastrointestinal tract as well as other sarcomas of the gastrointestinal tract and a metastatic melanoma to the ileum (misdiagnosed as a stromal tumor), were kit receptor negative.

An interesting observation was the focal, diffuse proliferation of kit positive cells in the muscle wall at a distance from the tumors. Whether this represents focal ICC hyperplasia or a preneoplastic stage with proliferation of stem cells differentiating toward an ICC phenotype is unclear. The association of these hyperplastic areas with nuclear atypia suggests the latter possibility. This finding could explain the occurrence of multicentric tumors and tumor recurrences in the gastrointestinal wall distant from the primary tumor.[1,2,4]

CD34 identifies the majority of stromal tumors[42–44] and aids in their distinction from gastrointestinal leiomyomas and schwannomas, which are CD34 negative.[38] Similar to

previous reports, 56 of 78 stromal tumors in our series were strongly CD34 positive. The basis for CD34 positivity in stromal tumors is unclear. CD34 antigen, an 11-kd glycosylated transmembrane protein, was originally described as a hematopoietic stem cell marker.[45] However, immunoreactivity for CD34 has been observed in a wide range of normal tissues and tumors, including endothelium and various vascular tumors, peripheral nerve sheath tumors, localized fibrous tumors of various sites, dermatofibrosarcoma protuberans, and epithelioid sarcoma among others.[45] Our observation of CD34 positive delicate spindle cells, which are also kit positive in consecutive tissue sections and arranged around Auerbach's plexus, suggests that a subpopulation of ICC is CD34 positive. This finding could explain the CD34 immunoreactivity seen in most stromal tumors and also explain the dual kit/CD34 immunoreactivity in the areas of focal, diffuse, atypical ICC proliferation. The ICC directly associated with Auerbach's plexus have been referred to as ICC type I.[11] It is possible that the CD34 positive stromal tumors display an ICC type I phenotype while the CD34 negative tumors represent those with an ICC II to IV phenotype.

The embryonic origin of the ICC has remained unclear and disputed since their discovery more than 100 years ago. They have been considered as neural or glial and thus of neural crest origin as well as fibroblastic or smooth muscle in nature.[11] Based on the quail-chick marker system in which chimeric bowels were created by isotopically grafting quail vagal neural crest into chick embryos, the kit positive ICC were believed to be mesodermal in origin and to develop independently of enteric neurons with which they later established an anatomical and functional relation.[46] The intermediate filament profile of GIPACT, which are vimentin positive (78 of 78) and lack neural crest markers (0 of 78 S-100 protein; 0 of 78 neurofilaments), is compatible with mesodermal differentiation.

Although the kit receptor has been found to be essential for the normal development and proliferation of gastrointestinal pacemaker cells,[19,21–23] nothing is known regarding its function in the neoplastic transformation of pacemaker cells or its role in proliferation and progression of GIPACT. However, it is noteworthy that point mutations in the kit gene, causing ligand-independent activation of kit product, have been reported in malignant mastocytosis,[47,48] which originates from another kit receptor dependent cell system. A point mutation has also been identified in another closely related gene, colony-stimulating factor-1 gene, in a subset of acute myeloid leukemias.[49] Deletion of the extracellular domain and C terminus of the kit receptor has been associated with oncogene activation as well.[25] The kit gene product has been identified in some solid human tumors such as small cell lung carcinoma and pheochromocytoma.[50] Additional studies may unravel whether the kit gene plays any pathogenetic role in the development of GIPACT and their progression. Such information could be important in designing new diagnostic and treatment modalities and in predicting biological behavior.

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