Gastrointestinal tract spindle cell tumors with interstitial cells of Cajal: Prevalence excluding gastrointestinal stromal tumors

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Abstract. Leiomyomas and schwannomas of the gastrointestinal tract (GIT) are mainly comprised of spindle-shaped tumor cells and should always be differentiated from gastrointestinal stromal tumors (GISTs). Mast/stem cell growth factor receptor Kit (KIT) and discovered on GIST-1 (DOG1) are well-known diagnostic markers for the detection of a GIST by immunohistochemical staining. The aim of the present study was to assess the prevalence and significance of spindle cell tumors of the GIT with KIT- or DOG1-positive spindle-shaped cells, presumed to be interstitial cells of Cajal (ICCs), other than GISTs. A total of 71 leiomyomas and 35 schwannomas were examined and clinicopathological information was obtained. KIT and DOG1 immunostaining was performed to determine the proportions of positive cells. Mutation screening of KIT exons 9, 11, 13 and 17, and platelet-derived growth factor receptor α (PDGFRA) exons 12 and 18 was performed in cases with a relatively high proportion of either KIT- or DOG1-positive cells. The frequency of leiomyomas and schwannomas with KIT- and DOG1-positive ICCs was 35.2% (25/71 cases) and 5.7% (2/35 cases), respectively. Among the esophageal leiomyomas with KIT- and DOG-positive ICCs (14/25; 56.0%), 5 leiomyomas involved the muscularis mucosa and 9 leiomyomas involved the muscularis propria. All gastric leiomyomas with KIT- and DOG1-positive ICCs (11/25; 44%) involved the muscularis propria. All schwannomas with an increased proportion of KIT- or DOG1-positive ICCs were of gastric origin. No KIT or PDGFRA mutations were detected in 7 leiomyomas and 2 schwannomas. In conclusion, the majority of leiomyomas and the minority of schwannomas in the GIT had a significant portion of KIT- and DOG1-positive cells. All of the tumors were located in the upper GIT, and could be present in the muscularis propria or muscularis mucosa. The tumors represented a non-neoplastic proliferation of KIT- and DOG1-positive cells in the GIT. Careful evaluation of KIT- or DOG1-positive cells in spindle cell tumors of the GIT can assist in forming the correct diagnosis by differentiation from a GIST.

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Introduction

Mesenchymal tumors of the gastrointestinal tract (GIT) are mainly comprised of a spectrum of spindle cell tumors, including gastrointestinal stromal tumors (GISTs), leiomyomas, schwannomas, inflammatory fibroid polyps, fibromatoses, and more rarely, leiomyosarcomas. GISTs should always be considered in the differential diagnosis of spindle cell tumors of the GIT, as GISTs constitute the largest subset of mesenchymal tumors of the GIT and GIST cell morphology is usually spindle-shaped (70%), although certain GISTs consist of epithelioid cells (20%) or a mixture of cells (1,2). GISTs may be defined as mesenchymal tumors of the GIT that usually express the mast/stem cell growth factor receptor Kit (KIT) protein and harbor mutations of a gene that encodes for KIT or platelet-derived growth factor receptor α (PDGFRA) and probably originate from the interstitial cells of Cajal (ICCs) (3). Since KIT and PDGFRA tyrosine kinase inhibitors have become available, the proper identification of GISTs has become clinically important (4,5). Therefore, it is necessary to differentiate other mesenchymal tumors of the GIT from GISTs, particularly those consisting of spindle-shaped tumor cells.

KIT and DOG1 immunohistochemical staining is a well-known diagnostic tool for detecting GISTs (6,7). Notably, certain lesions among the mesenchymal tumors of the GIT, other than GISTs, are represented by a substantial number of KIT-positive or DOG1-positive spindle-shaped cells, presumed

to be interstitial cells of Cajal (ICCs). If this finding is obtained in a small biopsy, tumors with a high proportion of KIT- or DOG1-positive spindle-shaped cells can mimic a GIST.

The present study assessed the incidence and significance of KIT- or DOG1-positive spindle-shaped cells in leiomyoma or schwannoma of the GIT, which is the main differential diagnosis of spindle cell tumors of the GIT.

Materials and methods

Patients and tissue samples. Samples of the spindle cell tumors of the GIT, coded as leiomyoma or schwannoma, were obtained by polypectomy and endoscopic or surgical resection performed at Pusan National University Yangsan Hospital (Yangsan, Gyeongsangnam, South Korea) or Pusan National University Hospital (Busan, South Korea) between 2009 and 2014. A total of 106 patients (mean age, 32 years; range, 18-84 years) were examined based on the availability of material and clinicopathological information. The present study was approved by the Institutional Review Board at Pusan National University Yangsan Hospital after obtaining written informed consent.

Clinicopathological data, including age, gender, tumor type, tumor size and tumor location, were recorded. Follow-up information was also recorded. The results of previously evaluated immunohistochemical staining for smooth muscle actin (SMA), desmin and S100 protein were obtained from pathology reports. Hematoxylin and eosin-stained slides were reviewed, and previously performed immunohistochemical staining for SMA, desmin and S100 protein was reassessed. Table I provides an overview of the main clinicopathological features.

Immunohistochemistry. In all 106 cases, additional KIT and DOG1 immunostaining was performed to determine the proportions of positive cells within leiomyomas or schwannomas of the GIT. Rabbit polyclonal anti-human CD117/c-KIT antibody (1:600 dilution; Dako, Carpinteria, CA, USA) and rabbit monoclonal anti-DOG1 (SP31) antibody (1:500 dilution; Cell Marque, Rocklin, CA, USA) were used for immunostaining. Immunohistochemistry was performed on serial 4-μm thick, formalin-fixed paraffin-embedded (FFPE) blocks using the EnVision Detection system (Dako) with diaminobenzidine as the chromogen, according to the manufacturer's protocols.

Immunohistochemical staining was interpreted by two independent pathologists. KIT and DOG1 immunostaining was assessed over the range of 0-100% positive staining of cells. Staining of the sections with antibodies against KIT and DOG1 was considered significant when >5% of the cells within the tumor were positive.

Analysis of c-Kit and PDGFRA gene mutations. Mutation screening of KIT exons 9, 11, 13 and 17, and PDGFRA exons 12 and 18 was performed in selective cases with a relatively high proportion of either KIT- or DOG1-positive cells (>15%).

DNA was extracted from five 10-μm thick FFPE sections containing a representative portion of tumor tissue using the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. DNA (50 ng) was amplified in a 20-μl reaction solution containing 10 μl of 2X concentrated HotStarTaq Master mix (Qiagen), including polymerase chain reaction (PCR) buffer with 3 mM $MgCl_2$, 400 μM of each dNTP and 0.3 μM of each primer pair. PCR products were gel purified on 2% gels with a QIAgen gel extraction kit (Qiagen). DNA templates were processed for the DNA sequencing reaction with forward and reverse sequence-specific primers using the ABI-PRISM BigDye Terminator ver. 3.1 (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). Sequence data were generated with the ABI PRISM 3100 DNA Analyzer, and sequences were analyzed by Sequencing analysis 5.1.1. software (both Applied Biosystems; Thermo Fisher Scientific Inc.) to compare variations.

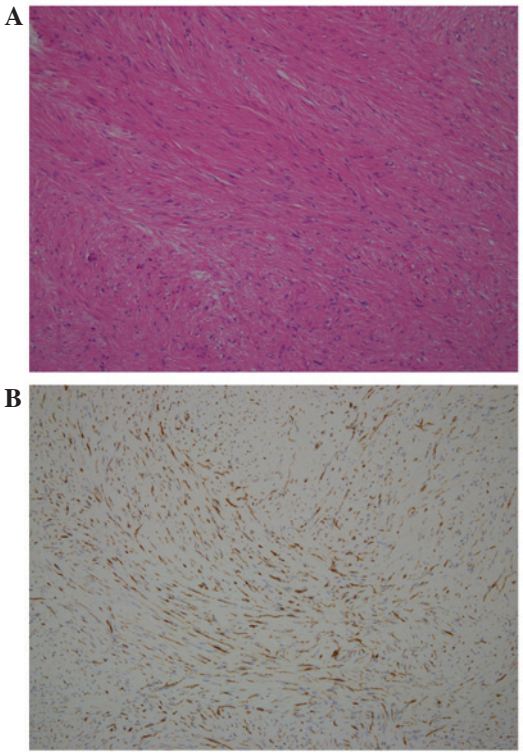

Figure 1. Leiomyoma. (A) Leiomyoma composed of intersecting fascicles of spindle-shaped smooth cells with eosinophilic cytoplasm (hematoxylin and eosin staining; magnification, x100). (B) An immunohistochemical stain for DOG-1 showing a high density of DOG1-positive interstitial cells of Cajal within the tumor (magnification, x200). DOG1, discovered on GIST-1.

Results

The study population was comprised of 71 cases of leiomyoma and 35 cases of schwannoma. The age and gender distribution of the 106 cases with pathological data (tumor site, location, size and results for SMA, desmin and S100 protein) is shown in Table I. Leiomyomas were more common in the esophagus and schwannomas were more frequent in the stomach. The majority of leiomyomas and schwannomas originated from the muscularis propria. Leiomyomas measured 0.2-11.0 cm in their greatest dimension (median, 1.9 cm), and schwannomas measured 0.5-8.0 cm (median, 2.5 cm). All masses included in this study were well circumscribed and were composed of spindle cells that did not show nuclear atypia or pleomorphism. Mitoses were absent or few in number. Leiomyomas (Fig.1)

Table I. Clinicopathological characteristics of the spindle cell tumors of the gastrointestinal tract.

Characteristic	Leiomyoma (n=71)	Schwannoma (n=35)
Mean age, years	34	27
Gender ratio (male:female)	37:34	13:23
Mean tumor size, cm	2.6	3.0
Tumor distribution		
Esophagus		
MM	15	0
MP	20	2
Stomach		
MM	3	2
MP	18	25
Small intestine		
MM	0	0
MP	2	0
Colon and rectum		
MM	10	3
MP	3	3
Immunohistochemistry		
SMA	All diffusely-positive	All negative
Desmin	All diffusely-positive	All negative
S-100 protein	All negative	All diffusely-positive

MM, muscularis mucosa; MP, muscularis propria.

Table II. Spindle cell tumors of the gastrointestinal tract with mast/stem cell growth factor receptor Kit- and discovered on GIST-1-positive interstitial cells of Cajal.

Characteristics	Leiomyoma (n=25)	Schwannoma (n=2)
Mean age, years	49.5	62
Gender ratio (male:female)	13:12	0:2
Mean tumor size, cm	3.1	2.8
Tumor distribution		
Esophagus		
MM	5	0
MP	9	0
Stomach		
MM	0	0
MP	11	2

MM, muscularis mucosa; MP, muscularis propria.

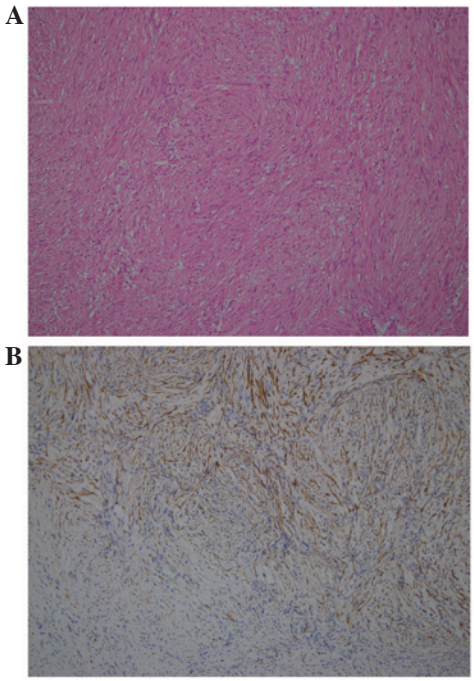

Figure 2. Schwannoma. (A) Schwannoma composed of short bundles of spindle-shaped Schwann cells (hematoxylin and eosin staining; magnification, x100). (B) In a few cases, the number of discovered on GIST-1-positive interstitial cells of Cajal were significantly increased (magnification, x200).

showed intersecting fascicles of spindled cells that closely resembled normal smooth muscle cells with eosinophilic cytoplasm (Fig. 1A) and schwannomas (Fig. 2) were composed of spindled cells with elongated, wavy nuclei (Fig. 2A). Leiomyomas were diffusely-positive for SMA and desmin, and negative for S100 protein, whereas all schwannomas showed completely opposite patterns (data not shown). None of the patients were reported as experiencing tumor recurrence.

KIT immunoreactivity was usually diffuse and cytoplasmic, but occasionally it showed a membranous or a dot-like pattern, and DOG1 staining appeared predominately localized to the plasma membrane (Figs. 1B and 2B). Mast cells were identified in all KIT and DOG1 immunohistochemically-stained slides. In addition to the mast cells, the slides showed a second population of KIT- or DOG1-positive cells. These cells were easily differentiated from mast cells based on their spindle shape and long dendritic processes typical of ICCs. In general, these cells were diffusely or sparsely distributed throughout the tumors, but focal aggregates were identified in certain cases. There was no significant ICC hyperplasia in the adjacent muscle layers in all cases.

The proportion of KIT- and DOG1-positive ICCs varied in leiomyomas from 0 to 30% of the lesional cells. The frequency of leiomyomas with a significant proportion of KIT- and DOG1-positive ICCs was 35.2% (25/71 cases) (Fig. 1B). Of these 25 cases, 14 (56.0%) were located in the esophagus and 11 (44.0%) were located in the stomach. Among the esophageal leiomyomas with KIT- and DOG1-positive ICCs (14/35, 40.0%), 5 leiomyomas involved the muscularis mucosa and 9 leiomyomas involved the muscularis propria. All gastric leiomyomas with KIT- and DOG1-positive ICCs (11/21, 52.4%) involved the muscularis propria. By contrast, few KIT- or DOG1-positive ICCs or no expression was detected in the majority of schwannomas, with the exception of 2 cases in which 15 and 20% of the lesional cells were KIT- and DOG1-positive ICCs, respectively (Fig. 2B). All these tumors were gastric shwannomas arising from the muscularis propria (Table II).

Mutation analysis of KIT exons 9, 11, 13 and 17, and PDGFRA exons 12 and 18 was performed in the 7 leiomyomas and 2 schwannomas that exhibited >15% KIT- and DOG1-positive lesional cells. No KIT or PDGFRA mutations were detected in any of these 9 cases (Fig. 3).

Discussion

The present study examined the incidence and distribution of KIT- or DOG1-positive cells in spindle cell tumors of the GIT, and assessed their true nature by performing KIT and PDGFRA mutation analysis to rule out the possibility of a GIST. Leiomyomas and schwannomas of the GIT mainly exhibit spindle cell morphology and should always be differentiated from a GIST. KIT- and DOG1-positive cells in spindle cell tumors of the GIT represent ICCs, as these cells closely resemble normal ICCs with their dendritic-like processes and are reactive to KIT and DOG1, which are robust ICC markers (8). The present study found that these cells were present in gastric schwannomas (8%), as well as in esophageal (40%) and gastric (52.4%) leiomyomas, particularly in the muscularis propria. The cells are diffusely distributed or focally aggregated.

Several studies have noted KIT- or DOG1-positive cells in leiomyomas of the GIT, which were interpreted as a colonization of Cajal cells between smooth muscle cells (4,8,9). Deshpande *et al* (9) reported that a significant number of upper deep leiomyomas of the GIT possessed KIT- or DOG1-positive ICCs, and an increase in the numbers of KIT- or DOG1-positive ICCs within a tumor raised doubt as to whether the mass was a GIST. Paying careful attention to the morphological appearance of these cells, as well as the tumor cells, can assist in establishing the differential diagnosis of a GIST, particularly in a small biopsy due to the clinical relevance. The present study also observed that KIT- or DOG1-positive ICCs were absent in the lower GIT. However, the study showed that leiomyomas that arose from the musularis mucosa also possessed a significant proportion of KIT- or DOG1-positive ICCs, particularly in the esophagus (5/15, 33.3%). KIT- or DOG1-positive cells could be distinguished by their long and slender dendritic-like processes eminating from elongated and plump smooth cells with blunt-ended nuclei and an eosinophilic cytoplasm.

By contrast, 2 cases of gastric schwannomas in the GIT exhibited KIT- or DOG1-positive ICCs and each possessed a significant proportion of these cells. Previous studies have reported the absence of KIT-positive cells in schwannomas of the GIT (10,11). Schwannomas of the GIT exhibit morphological clues that can aid in making a diagnosis, such as wavy Schwann cells with a tapered end and occasionally prominent lymphoid cuffs in the periphery (10), even if there is an area with a high density of KIT- or DOG1-positive cells, particularly in resected specimens. However, we suggest that gastric schwannoma should be considered if a spindle cell tumor is detected on a small biopsy from a gastric lesion when a gastrointestinal mesenchymal tumor is clinically suspected.

Several hypotheses have been described for explaining the presence of ICCs: i) Infiltration and hyperplasia by non-neoplastic ICCs from the adjacent muscularis propria, in which KIT- or DOG1-positive ICCs are normally detected; and ii) a type of stromal tumor with mixed differentiation, including ICCs, smooth muscle and neural elements (8). In the present study, it was found that the distribution of KIT- or DOG1-positive ICCs was occasionally diffuse throughout leiomyomas and schwannomas and not prominent at the peripheral portion of the tumors. There was no significant ICC hyperplasia in the adjacent tissue in all cases. Furthermore, the absence of KIT and PDGFRA mutations in cases of tumors with a significant proportion of KIT- and DOG1-positive ICCs supports the notion that these cells were not true neoplastic GIST cells, but that they were non-neoplastic ICCs between tumor cells.

In conclusion, the majority of leiomyomas and the minority of schwannomas in the GIT have up to 30% of all lesional cells formed from KIT- and DOG1-positive cells, respectively. KIT- and DOG1-positive cells have dendritic-like processes closely resembling normal ICCs and are reactive to the markers of ICCs, namely, KIT and DOG1. All of the tumors were located in the upper GIT, and moreover, all schwannomas with increased proportions of KIT- or DOG1-positive ICCs were of gastric origin. The tumors could be present in the muscularis propria or muscularis mucosa. Furthermore, KIT and PDGFRA mutations were absent in these tumors. It was concluded that these tumors represent a non-neoplastic proliferation of KIT- and DOG1-positive cells in the GIT. The presence of a large numbers of these cells prompts the diagnosis of a GIST, particularly in limited biopsy samples. Careful evaluation of KIT- or DOG1-positive cells in spindle cell tumors of the GIT that are located in the muscularis proper or mucosa can

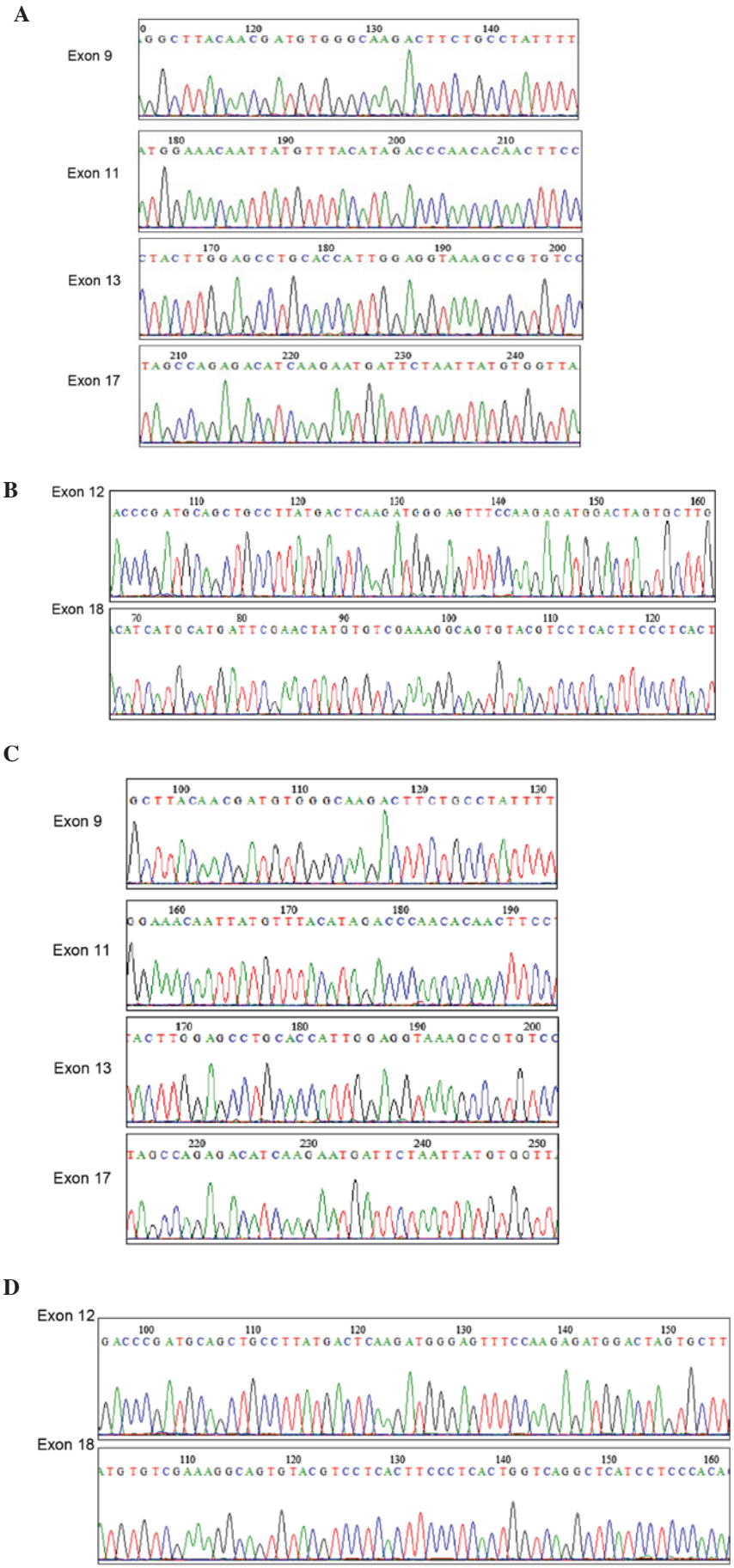

Figure 3. KIT and PDGFRA mutation analyses on representative cases. (A) KIT exon 9, 11, 13 and 17 mutations are absent in leiomyoma. (B) PDGFRA exon 12 and 18 mutations are absent in leiomyoma. (C) KIT exon 9, 11, 13, and 17 mutations are absent in schwannoma. (D) PDGFRA exon 12 and 18 mutations are absent in schwannoma. KIT, mast/stem cell growth factor receptor Kit; DOG1, discovered on GIST-1; PDGFRA, platelet-derived growth factor receptor α.

aid in making the correct diagnosis by differentiating the tumors from a GIST.

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